Hann, Harry Wilbur
1927. The history of the germ cells of Cottus bairdii Girard
Jour. Morph. and Physiol., v. 43, no. 2, Mar. 5

Cytology, germ cells — Cottus bairdii Girard (fish)
Oogonia, Spermatogonia } origin and development

The Wistar Institute Press
Philadelphia, Pa., U.S.A.

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A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the University of Michigan.

THE HISTORY OF THE GERM CELLS OF COTTUS BAIRDII GIRARD [1]

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EIGHT PLATES (SEVENTY-SEVEN FIGURES)

AUTHOR'S ABSTRACT

The primordial germ cells are derived from entodermal giant cells which are found first before the gut is formed and later along the ventral and lateral margins of the gut. Some of these cells pass through the lateral mesoderm to a position dorsal to the gut, where they are distinctly recognizable as germ cells. They are then shifted to the gonad region.

Sex could be distinguished first at fifty-two days. The female sex is indicated by early maturation stages and the beginning of the oviducal groove. The male sex is distinguished by the presence of the sperm duct. No tendency toward juvenile hermaphroditism is apparent.

A portion of the oocytes formed during the first season matures for the first spawning, which takes place at the age of two years. The remainder form a reserve supply, which is increased each year by oocytes formed from dormant oogonia.

Maturation in the male begins in September and continues until spawning-time in April. Spermatogonia lying dormant within the cysts during maturation give rise to the sperm of the next season.

'Spermatid masses,' probably formed by the fusion of spermatids, are found in the cysts with the ripening sperm.

Definitive sex cells in both sexes have their origin only in primordial germ cells. No transition from somatic cells to germ cells was found at any stage.

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INTRODUCTION

Work on the history of vertebrate germ cells dates back to Waldeyer ('70) who first observed the cells in the germinal epithelium, from which he supposed that they were derived.

[1] Contribution from the Zoölogical Laboratory of the University of Michigan.

Nussbaum ('80) expressed the view that the germ cells were segregated early in the development of the embryo, and were not derived from somatic cells. This view gained support from work on certain invertebrates (Ascaris and others), where the germ cells were definitely traced back to early segmentation stages. Weismann's germ-plasm theory gave an impetus to investigations of this problem, but from the vertebrate side the results have been more or less conflicting. A few investigators, including Rückert ('88) and van Wyhe ('89), saw definite segmented regions in the mesoderm to which they ascribed the name 'gonotome,' and from which they believed that the germ cells were derived. This view has not received much support. It is generally admitted that the so-called primordial germ cells appear in the early embryonic stages, and, by the work of B. M. Allen ('07, 09, and '11) and others on species from various vertebrate groups, it seems certain that these cells, when first recognizable, are in the gut entoderm, and from there they migrate to the gonad region. Some workers (von Berenberg-Gossler, '14, and others) do not believe that these cells give rise to the definitive germ cells. Others admit that they may give rise to definitive germ cells in lower vertebrates, but that in higher vertebrates they degenerate, and that the definitive germ cells here are derived from somatic cells. Still others have thought that the first set of definitive germ cells are derived from the primordial germ cells, but that the subsequent sets are derived from somatic cells (Gatenby, '16, and others). Thus it appears that there is no unity of opinion regarding the origin of the definitive germ cells in vertebrates.

Another problem that has appeared in connection with the development of germ cells, especially in the lower vertebrates, is that of a tendency toward a juvenile hermaphroditism at the time of sex differentiation. Brock ('81) found that this tendency was prevalent in certain teleosts during their young stages, and that some teleosts were often hermaphroditic in the adult stage. A juvenile hermaphroditic condition has been definitely established for certain cyclo-

stomes (Okkelberg, '21, and others). In amphibians, too, this condition is of common occurrence. The question suggested itself as to whether or not this condition might be almost universal in lower vertebrates.

With these questions in mind, it seemed desirable to make a thorough study of the germ-cell history of one species of teleost, and for this purpose Cottus bairdii was selected, since it is fairly abundant in the smaller streams of Michigan and rather easily obtained during every part of the year.

Although considerable work has been done on isolated periods in the history of the germ cells of fishes, no consecutive account has appeared of the whole cycle. Incidental to the main problem, it was found expedient to make a study of the spawning habits of the species and to ascertain the rate of growth, so that the life-cycle of the animal might be correlated with the germ-cell cycle. Other problems that have come up in connection with the history of the germ cells will be taken up in their proper sequence.

I wish to acknowledge my indebtedness to Dr. P. O. Okkelberg, of the University of Michigan, for his aid in the supervision and criticism of the work, and also to Mr. Carl L. Hubbs, of the University of Michigan Museum, for helpful information and suggestions.

MATERIALS AND METHODS

Cottus bairdii is found quite abundantly in all of the smaller streams around Ann Arbor, Michigan, and specimens were obtained during every month of the year except January. Most of the material was obtained in Mallet Creek, a small tributary of the Huron River, about three miles east of Ann Arbor. A minnow seine of $\frac{1}{4}$-inch mesh was used for collecting the larger individuals, and a seine of $\frac{1}{8}$-inch mesh, either with or without a cheesecloth lining, was used in securing the fry. Eggs were easily found beneath stones, and collections of these were made semiweekly during the period of incubation. In a few cases artificially fertilized eggs were

used, and a few of the fry were hatched in the laboratory from eggs brought from the stream.

The larger specimens used for cytological work were brought to the laboratory and preserved at once. Usually the gonads were removed before fixing, though good material was obtained by opening the abdominal cavity and fixing the entire fish. Smaller specimens and eggs secured in streams were usually preserved as soon as caught. Bouin's solution gave good results as a fixative, and was used for most of the material. Allen's modification of Bouin's, Flemming's strong, Zenker's, and Champy-Kull's fixing solutions also were used with success. In case of the adult specimens, only the gonads, or parts of them, were embedded, while in the case of the smaller specimens, a part of the body was embedded together with the gonads. Sections were cut 6 μ, 8 μ, and 10 μ in thickness, stained in iron-hematoxylin, and counterstained either in Licht-grün or eosin.

The results are based on the examination and study of about 470 specimens taken at various times of the year.

BREEDING HABITS

1. Observations on the breeding habits of Cottus bairdii

a. Observations on individuals in the stream. Cottus bairdii is a bottom form, living usually under stones or vegetation, though it is not unusual to see individuals resting on the bottom without any protection at all. If one is disturbed, it darts away quickly, but goes only a short distance before stopping.

In specimens of three or more years of age the sex may usually be distinguished by the shape of the head, that of the male being broader, but in younger individuals this criterion is not reliable. The more prominent genital papilla of the male is also a sex-distinguishing character in mature individuals.

At spawning time, the male prepares the nest under a stone or some other favorable object. The nest consists only of a hole, which is provided with a suitable covering and

which can be easily protected against enemies. The nest is then visited by one or more females, and eggs are deposited on the under surface of the stone or other object which covers the nest. The female then leaves, and the male guards the nest throughout the incubation period. According to Mr. Carl L. Hubbs, Cottus bairdii, in the colder streams of northern Michigan, is more common in the dense patches of vegetation than under stones, and to some degree at least deposits eggs on the plants. In warmer streams he finds it more common under stones, particularly at breeding time.

During the breeding season, one may frequently find Cottus nests by carefully lifting the stones in a place where the current is rather swift. On April 19,1924, the first eggs of the season were found. Most of the females were still in a gravid condition at this date, however, and April 20th was taken arbitrarily as the spawning date for that year in calculating the age of young fishes. A difference in the stage of development of early embryos indicates that spawning lasts through a period of four or five days. The nest with eggs found on April 19th was observed again a few days later, and a second cluster of eggs had been added. Two or three clusters of eggs in a nest is a common occurrence, and in one nest five clusters were found. The nests were in water from 15 to 35 cm. deep. The depth changed somewhat with the rise and fall of the stream, but no nests were found to be left above water. In a favorable place in the stream several nests were often found near each other. It was the custom in collecting eggs to take samples of five clusters each time, and these were sometimes secured by following the stream for only a few meters.

The hatching date, like the spawning date, could not be set exactly, but May 26th was taken arbitrarily as the approximate date. Newly hatched fry were found in one nest at this time, and a few eggs in three other nests. On May 28th, no eggs were found. Considering April 20th as the approximate spawning date and May 26th as the approximate hatching time, the incubation period for 1924 in this particular stream

was thirty-six days. The incubation period varies, of course, in different years, depending upon the average temperature of the water. The temperature during the season differs with the time of day and changes of weather, so that an average temperature would be difficult to calculate. Readings taken in the morning usually showed a temperature of from 5° to 9°C., but during the day sometimes the readings were as high as 16°C. Eggs kept in the laboratory in warmer water hatched in twenty days. Toward the close of the incubation period, eggs taken from the stream and carried in fresh water to the laboratory hatched out on the way, evidently as a result of the agitation in carrying them.

The nests apparently are abandoned soon after hatching time. In the nest where newly hatched fry were found on May 26th, the male was still present on May 28th, but only one young fish was found in the nest. Several fry were taken in a fine net on May 28th and at frequent intervals thereafter. These were usually caught by dragging the net over a bed of smooth pebbles.

b. Observations on individuals in the laboratory. Cottus bairdii may be kept quite easily in an aquarium provided with running water and a stream of bubbling air. A few specimens were kept in this way for nearly a year, and were then preserved. Attempts to keep them in still water did not prove successful, though they often lived for some time. Apparently, they require more oxygen than many fish, necessitating some special precaution on this account. They are fond of earthworms, which formed the chief part of their diet while in captivity, but they also eat small fish, not even hesitating to devour the young of their own species.

A few days before spawning time in 1924, several pairs of Cottus bairdii from the stream were placed in aquaria, with the hope that their breeding habits might be observed. Stones, bricks, and pieces of crockery were placed in the aquaria, in order to make conditions as favorable as possible for spawning. In one instance the eggs were laid apparently in the normal manner, and this case will be described in some detail.

On April 12th, a 70-mm. male and a 52-mm. female were placed in an aquarium $8 \times 10\frac{1}{2} \times 16$ inches. For a shelter a piece of earthen flower-pot was placed in the aquarium, with the concave side down. A piece of tin which could be removed easily was kept over the exposed side of the shelter, on the outside of the aquarium, and observations were made several times daily. On April 23rd, about 1.30 P.M., the tin was removed, and the female found along one side of the nest with her ventral side up against the piece of crockery. The male lay close beside her, dorsal side up, apparently holding her in place with his body and pectoral fin. Both were headed toward the front of the aquarium, and the pectoral fins of the female almost concealed the eggs, which had been or were being deposited. After remaining in this position for about fifteen minutes the male turned and headed in the opposite direction. The female then soon righted herself and moved to the edge of the cover, leaving the cluster of eggs clinging to the roof of the nest. The male remained near the eggs, and once turned his ventral side toward them, but the emission of milt was not observed. This probably took place at the time the eggs were laid. The male continued to guard the eggs from this time on, while the female remained outside and paid no more attention to them. The temperature of the water in which the eggs were laid was 12.8°C., but two days later the aquarium was changed to another tap which registered about 15°C. In about twenty days several young hatched out, and the adults were removed to another aquarium for fear they might eat their offspring. The young were fed daily and continued to live for several weeks.

A number of other females laid eggs in the laboratory, but they all deposited them on the bottoms of the aquaria and deserted them. Two females, one just before laying, and another while still over the eggs, were seen to bend their bodies in a writhing manner, as if trying to extrude the eggs. The ovary of one female dissected just after egg-laying contained no mature eggs and another contained only one.

Several attempts were made to fertilize eggs artificially, and two of these proved successful. The eggs were removed from the female by pressing on the abdomen, and were mixed with bruised testis in a watch-glass. One batch kept in still water died before hatching, but the other, kept in running water, hatched about sixty young. The eyes showed through the egg membrane in twelve days, and hatching occurred in twenty days. The temperature of the water was from 13° to 15°C.

The fry still have large yolk sacs at hatching time, but these disappear in a few days and feeding begins. Chopped tubificid worms were fed to them daily, but this food was evidently insufficient, for fry kept in the laboratory did not thrive as well as those in the stream. Growth was slow and mortality high, and at the end of six weeks the cultures were abandoned.

2. Literature and discussion

Gage ('78), in his "Notes on the Cayuga Lake star gazer," described the nesting of Cottus near the shore of Cayuga Lake, New York. The subspecies dealt with was probably C. kumlieni, but the description might well be applied to typical bairdii, the subspecies used in the present report. He found the nests in water from 12 to 50 cm. deep. The fish seemed to have forethought, he supposed, in depositing the eggs in deeper water in May, so that the shallow water of July would not expose them. He found a fish accompanying the eggs in each case, but apparently did not observe that it was the male.

B. G. Smith ('22) gave a short description of the nesting habits of Cottus meridionalis (=bairdii). His observations were accurate and were made in the same locality where the present work was done. He found the round or oval egg masses on the under sides of stones during April and May. The eggs differed in size and color, due partly to parentage. He sometimes found two or three egg clusters in one nest, which he thought was due to more than one female spawning

with the same male. He found an adult Cottus in nearly every nest, and, in all cases examined, these were found to be males.

Observations on Cottus gobio, the common European species, throw considerable light upon Cottus bairdii, because of the similarity of the two forms. It has been suggested that Cottus gobio is identical with one of the American species, which all bear a close resemblance to each other, but this is very doubtful (Smitt, '92).

The nesting habits of Cottus gobio, according to Smitt ('92), were observed first by Marsigli in 1726. He said that the female prepared the nest under a stone, laid the eggs and deserted them, and that the male then guarded the nest until the young were able to care for themselves. Heckel and Kner, in 1858, according to Smitt, learned from fishermen of the Drave, a tributary of the Danube, that the male prepared the nest. This view has generally been held by observers since.

Fatio ('82) and Surbeck ('00) described the breeding habits of Cottus gobio more in detail, but neither knew how the eggs were deposited.

Fraenkel ('13) first observed the spawning of Cottus gobio in the laboratory. The female cleaned the stone where the eggs were to be deposited while the male kept other fishes away. The female then deposited the eggs on the vertical side of the stone, and the male extruded the milt over them. The male guarded the eggs during the incubation period, and hatching occurred in twenty-eight days at a temperature of 15°C.

Schreitmüller ('25) observed the spawning of Cottus gobio in the laboratory, also, during the season when most of the present work was done. The male prepared the nest by digging a hole under a stone. Prior to egg-laying the male and female turned their ventral sides together as if copulating, but the observer does not think that internal fertilization occurs. The female lay with her ventral side turned against a stone while the eggs were being extruded. The

incubation period was twenty-seven days at a temperature of 15.5°C. when the parent was present and two days longer when the parent was absent, although the temperature was slightly higher. According to Schreitmüller, the female guarded the eggs both in the laboratory and in an observed case in the stream.

There are differences in the observations made upon the breeding habits of Cottus gobio as indicated above. Most observers have thought that the male prepares the nest, though it is probable that the female may aid, in accordance with the accounts of Marsigli and Fraenkel. Schreitmüller is alone, however, in thinking that the female guards the nest. If his observations are correct, this duty must be performed in some cases, at least, by the female—a condition which was not found in Cottus bairdii. The incubation period of Cottus gobio, according to both Fraenkel and Schreitmüller, is about a week longer than that of Cottus bairdii at approximately the same temperature, namely, 15°C.

LIFE-CYCLE AND RATE OF GROWTH

Since it was necessary in the study of the germ-cell cycle to know something about the life-history of the fish, observations were made on its rate of growth in order to ascertain the age at which the germ cells ripen and spawning takes place.

The young Cottus at the time of hatching measures about 6.4 mm. in length. It grows very fast during the following summer months, and by the time the winter season sets in its length has increased to about five times that at hatching. Little growth takes place during the first winter, but the following spring brings on another marked increase in size, which continues to the second winter, when growth is retarded again. Sexual maturity is reached at the age of two years, as Fatio ('82) also ascertained for Cottus gobio. At this time a length of from 45 to 70 mm. is reached.

The length of the adult males is apparently a little greater than that of the females. The average of 102 males over 60

mm. in length was 73 mm., while that of sixty-nine females, also over 60 mm. in length, was 69.4 mm. The largest individual taken was a male of 113 mm. Measurements were made from the tip of the snout to the base of the caudal fin.

Four large collections of Cottus were made at different times of the year, and length-frequency curves were plotted for the study of growth, as shown in chart 1. The ordinates represent the numbers of individuals, the data being smoothed by a running average of five, and the abscissae represent the lengths in millimeters. Curve A represents 235 individuals taken on August 10, 1923; curve B represents 195 individuals taken October 31, 1923; curve C represents 207 individuals taken on December 22, 1923, and curve D represents 202 individuals taken the following spring on April 12th, 16th, and 19th.

In curve A of August 10th, the O group (that is, the young of the year) is represented with its mode at 22 mm. By October 31st (curve B), the mode of this group has moved to 31 mm. Growth is then retarded on account of cold weather, and the curve of December 22nd (curve C) shows only a slight increase in size. By the following April (curve D), the peak has moved to 34 mm. This group consists of immature animals one year old, and it forms a distinct unit (now group I, the fishes being in their second year). The older individuals are breeding at this time, which indicates that breeding takes place at the age of two years. Returning to curve A, the I group, on account of rapid growth, is overtaking the II group of the third year. The mode at 46 mm. is obviously that of the I group and the one at 64 mm. that of the II group. The mode at 56 mm. is probably due to an overlapping and piling up of the I and II groups. Curves B, C, and D are not very definite beyond the youngest-year group, but the next older group is probably represented by the modes as indicated on the plate. Older individuals were not taken in sufficient numbers to indicate clearly the average size of the year groups represented.

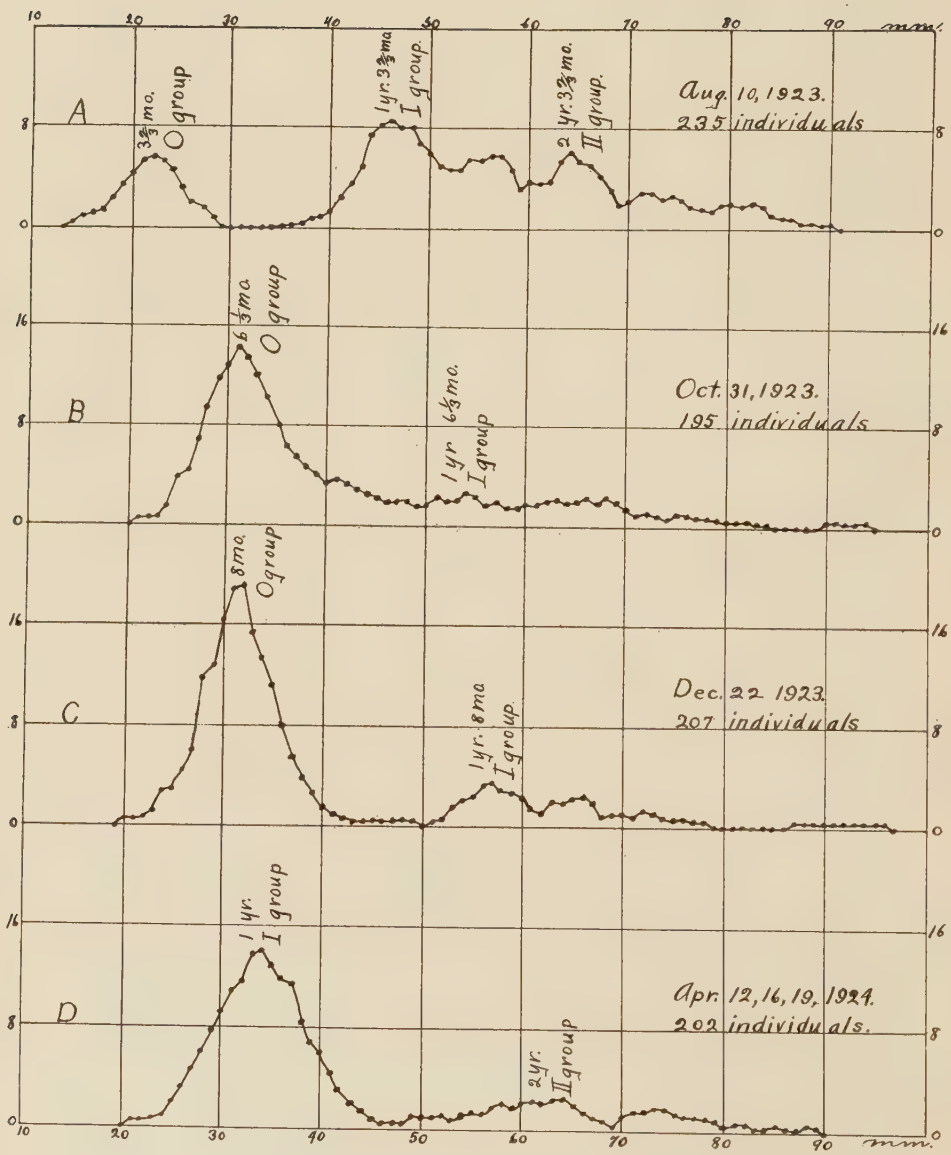


Chart 1 Length frequency curves for Cottus bairdii caught at different times of the year. The ordinates represent numbers of individuals, and the abscissae, lengths in millimeters. The curves are smoothed by a running average of five.

Another feature brought out in the curves is the relative number of young and adults. The young of the first year must suffer a high mortality, especially during the winter. A great decrease in the number of adults was also noted in the last year of collecting, presumably due to the intensive collecting in the small stream.

THE HISTORY OF THE GERM CELLS

The history of the germ cells may be divided into periods as shown in table 1. The indifferent period, during which the

TABLE 1

The germ-cell cycle in Cottus bairdii

	MALE	FEMALE
	Indifferent period. Origin, migration, and division of the primordial germ cells	
	Period of sex differentiation	
First year	Continued division of spermatogonia	Continued division of oogonia Formation and growth of oocytes
	Period of rest from division	Period of rest from division. Slow growth of oocytes
Second year	Division of spermatogonia	Division of oogonia. Formation and growth of oocytes
	Maturation, and formation of sperm for first spawning	Growth and maturing of oocytes for first spawning
	First spawning period	
Third year	Period of rest	Period of rest
	Division of spermatogonia	Division of oogonia. Formation and growth of oocytes
	Maturation and formation of sperm for second spawning	Growth and maturing of oocytes for second spawning
	Second spawning period	

sexes cannot be distinguished, and the period of sex differentiation have been most carefully studied. Attention has also been centered on the subjects of oogenesis and spermatogenesis, as well as on the origin of the subsequent generations of germ cells in both sexes after spawning.

A. The indifferent period, including the origin, migration, and division of the primordial germ cells

1. Observations on the early germ cells of Cottus bairdii. For the purpose of study, the indifferent period has been divided into two sections, the first dealing with the pre-germ-cell stages, particularly the origin and migration of the giant cells from which the germ cells are derived, and the second with the germ cells from the time they become clearly differentiated dorsad of the gut, up to the period of sex differentiation.

The individuals of the later stages of the indifferent period are designated both by their body length and computed age. In the early stages, however, only the body length is used, since it is much more accurate. As stated previously, the time of spawning varies as much as four or five days, hence the error in the computed age may be considerable in the young stages, since April 20th was taken for the spawning date in each case.

a. Pre-germ-cell stages. The primordial germ cells are derived from large cells of entodermal origin which correspond with what have been called 'giant cells,' on account of their large size. It seems well to use this term, since only a portion of these giant cells ever actually become germ cells.

Stages were studied covering the early development of the embryo as far back as the blastoderm stage. Not all of these stages can be described, but a representative series has been chosen which will indicate the origin of the primordial germ cells.

Embryo 1.05 mm. long. The nerve cord is forming at this time, the region being indicated by a difference in the arrangement of the cell nuclei. The periblast is separated

off as a layer between the embryo and the yolk in some places, but in others, particularly in the head region, it has not yet become differentiated. Where there is no separate layer, the periblast nuclei lie along the ventral surface among the cells of the embryo. Figure 1 is a transverse section of this embryo about the middle of the body. The periblast has separated off laterally, but not in the median portion. There is considerable variation in the size of the periblast nuclei, and in some places they appear to be dividing amitotically. There is no indication that these periblast cells ever form a part of the embryo, but they are mentioned here because they have been supposed by Reinhard ('24) to give rise to the primordial germ cells.

Embryo 1.64 mm. long. In this embryo the optic vesicles, the notochord, and Kupffer's vesicle are formed. Figure 2 shows a section at a little higher magnification than figure 1. This section contains several giant cells which are first distinguishable here, and extend only through a few sections. The entoderm is separated off from the surrounding tissue in the lateral, but not in the median portion of the section. The giant cells may be considered as undifferentiated cells of the entodermal layer. Large periblast nuclei in syncytial cells are quite common at this time. Three of these are shown in figure 2. Although the periblast nuclei are in close proximity to the giant cells, there is no evidence that the giant cells are derived from them.

Embryo 1.91 mm. long. At this stage the entoderm is a definite layer and contains many giant cells. These cells vary in shape from round to oval in cross-section, or, when crowded, they may appear rectangular or polygonal. The nuclei are nearly round and contain several nucleoli of varying sizes. Figure 3 shows the ventral portion of a transverse section about the middle of the body and a little laterad of the median line. The periblast is now a thick membranous layer. Two of its nuclei are shown in the figure. The cells of the lateral plate are crowded into a dense layer above the entoderm. The coelom has not yet formed, but in some places

the cells of the lateral plate are forming in double rows preparatory to coelom formation. In this embryo also, as well as in the two following stages, the giant cells are present as large, undifferentiated cells in the entodermal layer.

Embryo 2.04 mm. long (ten days old). In this embryo Kupffer's vesicle is no longer present. The optic cups are forming, but the lenses have not yet appeared. The coelomic cavity is present almost as far back as the giant cells are found, and in a few sections the excretory ducts are forming as evaginations from the coelom. The entodermal layer extends upward beneath the notochord to form the gut. With this the lateral entoderm is drawn toward the median line, leaving the lateral mesoderm in contact with the periblast nearly as far back as the giant cells extend. In the caudal part of the giant-cell area, where a few giant cells are found, the entoderm still extends laterad beneath the mesoderm. As a result of the gut formation and the retraction of the entoderm, the giant cells are shifted toward the median line, and are then found along the ventral and lateral edges of the gut. Apparently, at this time, many of the giant cells become differentiated and help to form the gut.

Figure 5 is a camera-lucida drawing of an embryo of this stage, showing the area in black where the giant cells are found. Figure 51 is a photograph of a transverse section at line *cd* in figure 5, showing the gut with the giant cells lying along the ventral and lateral margins. The gut has the appearance of being closed, but later stages show that the closure at this time, except in the anterior part of the giant-cell region, is only temporary, and that most of the giant cells soon leave this position. Just below the gut in the figure are some large periblastic nuclei.

The shape of the giant cells is round or oval, the outline regular, and the cytoplasm clear. The nuclei contain from two to six dark-staining nucleoli, which are often irregular in shape. It is impossible to distinguish clearly between the cells which are to form the gut and those which are to become germ cells, since they range in size from the small differen-

tiated cells of the gut to the large cells which are clearly undifferentiated.

Figure 52 is a photograph of a section through this embryo farther craniad, at line *ab* of figure 5. At the left side of the drawing are four giant cells which have become free from the gut and are ready to pass into the mesoderm. At this point, which is near the anterior end of the giant cell region, there are differentiated cells below the lumen of the intestine, and the gut is permanently closed. Some of the undifferentiated giant cells apparently remain in the gut entoderm and probably become differentiated there into tissue cells. One of these is shown at *x*.

Embryo 2.36 mm. long (ten days old). This embryo was taken from the same cluster of eggs as the one described above, but is a little further developed, as shown by its greater length, the farther ventral extension of the gut, and the presence of the excretory ducts through the whole length of the gonad region.

In the caudal two-thirds of the region which the giant cells occupied in the preceding stage, most of the cells are now found in the undivided portion of the lateral plate, between the excretory ducts and the lateral edges of the gut. Figure 53 is a photograph of one side of a transverse section, showing four of the cells. The gut is open on the ventral side through the region from which the giant cells have migrated except at the anterior end, and is pressed out flat against the periblast. This condition is apparently brought about by the pressure from above, due to the formation of the dorsal aorta, which results in a migration of the giant cells away from the ventral side. There is no indication that the cells move by ameboid movement. In the cranial one-third of the giant-cell region the cells remain below the gut and do not give rise to germ cells. They may help to form the gut or perhaps later become mesodermal cells. Two giant cells were found between the gut and the notochord in the caudal portion of the head region, but this is unusual. In the caudal portion of the giant-cell region a few cells still remain in the

concavity below the gut, from which position they could not easily shift.

b. Germ-cell stages. Embryo 2.77 mm. long. This embryo was taken from a batch of eggs in the laboratory, seven days after artificial fertilization. The gut is a closed tube and is flattened dorso-ventrally between the rest of the embryo and the yolk. At this stage the germ cells can be identified with certainty. They are round or oval, are larger than the surrounding cells, and stain less deeply. They lie dorsolaterad of the gut, between it and the dorsal aorta, or, in some cases, between the gut and the wolffian ducts. They are surrounded by small flattened mesodermal cells which have the appearance of later peritoneal cells, and from which at least a portion of the peritoneal cells develop. It is chiefly from these cells, also, that the somatic portion of the gonads develop.

There are nineteen germ cells in this embryo. Figure 54 is a photograph showing three of them. Obviously, not all of the giant cells which entered the mesoderm have become germ cells. These, in all probability, have become mesodermal cells, since no indication of degeneration was observed among them.

Embryo 3.21 mm. long. This embryo was taken from the same cluster of eggs as the preceding one, but is a little further developed. The gut extends farther ventrad and is more nearly cylindrical. The germ cells lie in about the same position as in the preceding stage, except that they are a little nearer the median line. There are twenty-one germ cells on this embryo. Figure 6 shows a transverse section containing three of them. The tissue in which the germ cells lie is now four or five cells in thickness. It is bounded on the dorsal side by the aorta, on the ventral side by the gut, and laterally by the wolffian ducts and the coelomic cavity.

Embryo 4.25 mm. long (fourteen days old). Figure 7 is a camera-lucida drawing of an embryo of this stage, showing the germ cell region in black, and figure 55 is a photograph

of a section at line *ab* in figure 7. At this stage the germ cells lie in a median position on the dorsal side of the gut. When the coelom formed dorsad of the gut, separation took place through the layer containing the germ cells, leaving most of them on the dorsal side. A few of the cells, however, are found within the mesentery itself, one of which is shown in the figure. Two germ cells also were observed lying ventrad of the mesentery, beneath the peritoneal covering of the gut. It is doubtful whether these ever would have reached the gonad region.

There is scarcely any difference in the relative position of the germ cells in the cranial and caudal parts of the gonad region, but the gut is a little farther separated by the coelom at the cranial end. There are about eighty germ cells in this embryo.

Embryo 4.7 mm. long (twenty days old). The germ cells at this stage are arranged bilaterally in the genital ridges, one ridge lying on each side of the dorsal mesentery. The tissue containing the cells is now only one or two cells in thickness and lies adjacent to the dorsal aorta. The germ cells in some places appear to be a part of the surface epithelium, but closer study shows them to be inside of the thin peritoneal cells. Where the germ cells are far apart in a longitudinal direction, the ridges are discontinuous, being formed only where the cells and their coverings protrude into the cavity. Figure 8 shows a transverse section through the genital region containing two germ cells, one in each ridge. There are fifteen germ cells in this embryo, nine on the left side and six on the right. The germ cells at this stage are more clearly distinguished from somatic cells than at any previous stage, since they remain in an undifferentiated condition, while the somatic cells are becoming more highly specialized.

Embryo 5.3 mm. long (twenty-four days old). The genital ridge at this stage is surrounded by a distinct layer of peritoneal cells, and it is being gradually constricted off from the region above. Figure 56 shows a transverse section through the genital ridges at this time, in which three germ

cells appear. In the mesentery is a blood vessel in which there are two blood cells. Other blood cells appear in the dorsal aorta above. The germ cells apparently are growing, and growth results soon after this in cell division.

Young 6.5 mm. long (thirty-six days old). This individual was taken from the creek just after hatching. At this stage secondary division of the germ cells was observed for the first time, one mitotic figure being found in this individual and two in another of the same size. Germ-cell clusters are found also, which is an indication of recent division. Figure 9 is a drawing of a transverse section about the middle of the gonad region. The germ-gland anlagen contain stroma cells now, and henceforth they will be called genital folds. The stroma cells are derived from peritoneal cells, and differ from them but little except in shape and position. They apparently migrate into the fold along the dorsal border. There is no evidence that mesenchyme cells enter the gonad with the germ cells. Follicle cells are found around the germ cells, and some of these have apparently retained their position from earlier stages, while new ones are formed either from peritoneal cells or from the forming stroma cells.

In this individual the gut lies laterad of the median line. One gonad is suspended free in the cavity from the dorsal wall of the coelom, while the other lies between the gut and the dorsal wall. The renal tubules are forming ventromediad of the wolffian ducts, but these appear only on one side in the section.

Young 7.3 mm. long (thirty-eight days old). The position of the genital folds is much the same as in the previous stage, one of them lying between the gut and the dorsal wall of the coelom and the other hanging free in the cavity. Figure 10 shows a cross-sectional drawing of the one which hangs free. The fold is suspended by a dorsal mesentery.

The genital fold has grown much, its cross-sectional area being about twice that of the preceding stage. Growth in the somatic portion of the fold is particularly noticeable near its attachment to the body wall. This portion is composed

chiefly of stroma cells, as shown in figure 10. There may still be an inward migration of peritoneal cells, although mitotic figures indicate that division is going on among the stroma cells as well as among the peritoneal cells.

Several mitotic figures are found among the germ cells at this stage, one of which is shown in figure 10. There is considerable variation in the size of the germ cells—probably a result of active growth and division.

Young 8.9 mm. long (forty-eight days old). Individuals of this stage are approaching the period of sex differentiation, but as yet no sex-distinguishing characteristics can be detected. The gonads vary greatly in shape, depending upon the surrounding structures. Some are triangular in cross-section, while others are oval or of other shapes. Blood vessels and blood cells appear in the gonad for the first time at this stage. They probably migrate in from the mesentery, since they are first found near it. The nuclei of the cells lining the blood vessels are smaller than other nuclei observed in the gonad, which may be evidence of an external origin.

Figure 11 shows a cross-section near the caudal end of a gonad of this stage. Several germ cells are shown, and surrounding them are numerous follicle cells forming capsular coverings for them. Often two or more germ cells are found together, forming a cell nest. These nests later become the tubules in the male, but they have only a transient existence in the female. The nuclei of the follicle cells are a little larger than those of the stroma cells. In some sections cell nests cannot be distinguished, and the germ cells with their follicle cells are scattered more or less evenly over the entire interior of the gonads.

The number of germ cells. The germ cells were counted as accurately as possible in several embryos, and the results are given in table 2. Embryos of the same age in this table are from the same egg cluster, but different ages represent different clusters. On the basis of the number of germ cells, the embryos may be divided into two groups. One group has from twelve to twenty-two germ cells and the other from

forty-nine to eighty. Furthermore, it may be noted from the table that the tendency at this stage to produce many or few germ cells is characteristic of all the embryos of the same cluster of eggs. No germ-cell division during migration was observed.

The embryo with only one germ cell must be considered as subnormal, and it is possible that some embryos may lack

TABLE 2

Table of germ cell counts. Embryos of the same age are from the same egg cluster, but embryos of different ages are from different clusters

EMBRYO	AGE	NUMBER OF GERM CELLS
1	7 days in the laboratory	19
2	7 days in the laboratory	21
3	14 days in the stream	80
4	14 days in the stream	64
5	17 days in the stream	19
6	17 days in the stream	18
7	17 days in the stream	1
8	20 days in the stream	15
9	20 days in the stream	22
10	20 days in the stream	12
11	27 days in the stream	49
12	27 days in the stream	70
13	27 days in the stream	63
14	31 days in the stream	14
15	31 days in the stream	18

germ cells entirely. One sterile adult was found, but its sterility was apparently due to parasitism.

The method of migration. No evidence of active migration of germ cells was observed. Migration is apparently brought about by a ventral shifting of the position of the gut and by the movements of other tissues during embryogeny.

Germinal epithelium. A germinal epithelium from which the germ cells take their origin is not found in Cottus bairdii. During the migration of the germ cells to the gonad region,

they become surrounded by cells of peritoneal origin, but there is no evidence that any of these cells ever become germ cells.

The early segregation of germ cells. The observations on Cottus bairdii support the theory of an early segregation of germ cells. The group of giant cells from which the germ cells take their origin also gives rise to entoderm and mesoderm cells as well, and only those giant cells which come to occupy a favorable position become germ cells. Since the germ cells have never become differentiated into somatic cells they may be said to take their origin in undifferentiated cells.

2. Literature and discussion. The method of early segregation and migration of germ cells in Cottus bairdii corresponds with the condition in other teleosts as found by most of the recent investigators. In the decade from 1880 to 1890, however, the opinions varied greatly with regard to these questions. Nussbaum ('80) found an early segregation of germ cells in the trout. Hoffman ('86) saw cells which looked like germ cells in the mesentery of Salmo salar, but did not know whether or not they were identical with the later germ cells. Jungerson ('89) was able to recognize the germ cells much earlier in some species than in others. In Zoarces he found them segregated in a 2-mm. embryo, in the same region which the giant cells occupied in the 1.98-mm. Cottus bairdii, as described above. In Rhodeus and other species he was unable to find them until they had reached the region of the germ-gland anlagen. Brock ('81) held to the germinal-epithelium theory for the muraenoids, and MacLeod ('81) held the same theory for Hippocampus.

The failure of some of these workers to find an early segregation of the germ cells may have been due to faulty technique, or perhaps to a lack of thorough investigation of the proper stages. Also the germinal-epithelium theory of Waldeyer ('70) was prevalent at this time, and probably influenced the opinions held by various workers. The investigators of the early germ-cell history in teleosts since 1890

all have found an early segregation of primordial germ cells, and the first recognizable germ cells are usually located in some part of the entoderm or the lateral mesoderm during the early stages of development.

Eigenmann ('91 and '96) traced the germ cells of Cymatogaster back to what he considered the fifth generation of segmentation cells. This is much earlier than they could be found in Cottus bairdii, but it is probable that the germ cells may be observed earlier in some species than in others.

Böhi ('04) found the primordial germ cells lying laterad of the wolffian ducts in a 3.14-mm. brown trout. In the salmon he found them in different parts of the lateral mesoderm at the 3.6-mm. stage. The peritoneal cells of the salmon, he thought, gave rise to 'indifferent cells,' 'follicle cells,' and 'germinal epithelium.' The indifferent cells also gave rise to follicle cells. The follicle cells, according to Böhi, did not differ from the other peritoneal cells, except as they were changed by pressure. The germinal epithelium, he thought, gave rise to germ cells between the 185th and the 227th days. The condition in Cottus bairdii is similar to the condition described by Böhi, in that the peritoneal cells give rise to follicle cells and the cells of the stroma, also in that the follicle cells may be formed from the stroma in early stages. There is no indication, however, in Cottus bairdii that the peritoneal cells ever give rise to germ cells. Böhi correlated this supposed transition with the increase in the number of germ cells near the end of the indifferent period; but this increase, as far as Cottus bairdii is concerned, needs no other explanation than rapid mitosis will supply.

Federow ('07) traced the germ cells in the brown trout to an earlier stage than Böhi did, finding them chiefly in the splanchnopleure.

Dodds ('10) found the primordial germ cells segregated in Lophius before the entoderm had separated from the mesoderm. When the layers separated, they lay in the lateral mesoderm, and later passed through the myotome to the gonad region. The chief distinguishing characters of the

primordial germ cells he gave as size and appearance, constancy of number, and correspondence of position. These characters are probably as definite as any which can be given.

Sink ('12) found the first germ cells of the toadfish, Opsanus tau, in a 3.5-mm. embryo. Some of the germ cells were already in the genital ridge at this time, others were still in the gut entoderm, while still others were in positions between these.

Miss Bachmann ('14) in Ameiurus nebulosus, found the germ cells first in the undivided lateral mesoderm of a 3.2-mm. embryo. When the coelomic cavity formed, they lay in the splanchnopleure. From here they migrated to the mesentery and then to the germ-gland anlagen.

Richards and Thompson ('21) found the germ cells of Fundulus first in a forty-six-hour embryo. At this time they were in the peripheral entoderm. They then moved through the lateral mesoderm to a position dorsal to the gut, then laterad to the gonad regions.

Essenberg ('23), working on Xiphophorus helleri, found the primordial germ cells taking up a bilateral position when the embryo was 3.2 mm. in length. Before this, they lay in a median position. Primordial germ cells, he thought, did not give rise to definite sex cells. In the female they disintegrated, and in the male remained functionless. Definitive sex cells in both sexes, he thought, came from peritoneal cells. No such degeneration, lack of function, or transition of the primordial germ cells was found in the present work.

Reinhard ('24) believed that the primordial germ cells of Scardinius had their origin in large cells which he called 'giant cells' (Riesenzellen). These cells, he thought, formed in the periblast from periblastic nuclei, then migrated into the mesoderm by ameboid movement. In Cottus bairdii no such origin of giant cells was found, although stages as early as the blastoderm stage were examined carefully. Periblastic nuclei were found in close proximity to the giant cells, but there was no indication of a transition. Jungerson ('89) thought that the periblastic nuclei formed somatic cells

which became incorporated in the ventral wall of the gut. Wilson ('89), however, considered the function of the periblastic nuclei as unknown. The formation of embryonic cells from periblastic nuclei, according to Wilson, was an old and disputed question reaching as far back as Kupffer's work in 1868, but he concluded that they took no share in the formation of the embryo. Reinhard's term 'Riesenzellen,' or 'giant cells,' is a very appropriate one, however, and has been adopted in this paper for the cells preceding the definitive germ cells, although observations in Cottus do not support his view as to their origin.

Allen ('07) found in the turtle Chrysemys that embryos from the same parent might have a relatively low or high number of germ cells, as in Cottus bairdii. He thought that the cause might be congenital or might be due to the time of the breeding season, but was not due to a difference in the stage of development. In Cottus bairdii the cause is apparently congenital.

B. The period of sex differentiation

1. General statement. During this period the sex of the young fish becomes established. If the sex of the animal is predetermined by an unequal distribution of sex-chromatin material at the time of fertilization, one should expect that the sexes would be quite distinct from the time sex characters first appear. This apparently is the case in some species, but in others there is a distinct tendency toward a juvenile hermaphroditic condition, for example, in the eel (Grassi, '19) and in the rainbow trout (Mršić, '23), indicating that the sex characteristics of one sex are borne at least in part by the other. A similar condition has been found in cyclostomes (Okkelberg, '21), and in amphibians (Hertwig, '05), and others. The literature bearing on this subject will be discussed later.

The early sex-distinguishing characteristics are confined to the structure of the germ gland itself, the structure of the germ cells, and the formation of ducts leading from the glands.

2. Development of the female. Female 9.5 mm. long (fifty-two days old.) At this stage the female sex is distinguished from the male by an oviducal groove on the ventral surface of the ovary, and also by the beginning of maturation phases among the germ cells.

The oviducal groove is an invagination which later closes up and forms the ovarian cavity and duct. It is usually on the ventral surface of the ovary, but it may occur on its lateral surface as well. The ovaries, like the testes and early indifferent glands, are not uniform in shape, and neither are the oviducal grooves. The grooves of the two ovaries unite caudally into a single groove, which upon closing forms the oviduct. Thus the oviduct and ovarian cavity become lined by peritoneum—a condition which has no homologue in the male.

In figures 12a, b, and c is represented a series of three drawings made from transverse sections of a pair of ovaries at this time. Figure 12a is a section through the anterior portion where the grooves are already closed. Only one germ cell appears in this section, as it is very near the cranial end. The lack of uniformity in the shape of the two ovaries and ovarian cavities is well shown. Figure b is a section through the middle of the ovaries where the grooves are still open, and c is a section through the posterior united portion where the common groove is also open.

Figures 13a, b, and c are outline drawings of the ovaries of another specimen of the same age. Figure a is from the anterior end, showing one groove closed and the other still open; b is from the middle, and c is from the posterior end, just craniad of the point of union of the two grooves.

The glands show a marked increase in size over those of the last stage, and also a correspondingly larger number of germ cells. Cell nests and frequent mitotic figures indicate that division is taking place rapidly. Large and small oogonia, oogonial divisions, synizesis stages, and growing oocytes are common. These stages will be described in more detail under the topic of maturation. The cell nests appear

to break up by an ingrowth of follicle cells between the germ cells. Cell nests are frequently found, but apparently only after rapid division.

Female 13.2 mm. long (fifty-nine days old). Figures 57a, b, c, and d represent a series of photographs of transverse sections through a pair of ovaries of this stage. Figure a is a section near the anterior end where the cavities are closed; figure b is a section through the middle where they are still open grooves; figure c is through the posterior closed portion where the cavities are separate, and figure d is through the united oviducal region. Figure 14 shows in outline a section of another pair of ovaries of this age, one of which has the cavity closed except at the point shown in the drawing, which is about the middle of the gland.

The germ cells are in much the same condition as in the previous stage. Early maturation stages are found, and many germ cells are still in the oogonial stage. Some of the primary oocytes show growth, but this takes place more rapidly in the next stage. Many germ cells lie close together in cell nests, while others are scattered, with follicle and stroma cells among them. Germ cells are found all through the dorsal portion of the ovary, and are not confined to the region next to the cavity.

Female 15.3 mm. long (seventy-three days old). The ovary is now in the form of a closed tube, and the germ cells lie within its dorsal portion. Transverse lamellae are beginning to form and are marked by fissures in the dorsal wall of the ovary. Figure 58 is a photograph of a longitudinal section of this ovary, showing the transverse fissures and the segments between them which will form the lamellae. The fissures occur at rather regular intervals, and are formed by a dorsal ingrowth of the epithelial lining of the ovary. Following the formation of the fissures, the lamellae enlarge and protrude into the ovarian cavity. Cell nests do not play the same important part in the formation of the ovary that they do in the testis. The oviduct, as shown in figure 58, is near the ventral side of the ovary—a position which it permanently retains.

Many of the oocytes at this time show a marked increase in size, due to the accumulation of yolk. The nucleus also becomes greatly enlarged. Figure 59 is a photograph of a section from another individual taken under higher magnification than figure 58. One of the large oocytes is shown, and some smaller ones in synizesis. Oogonia also appear, both in the resting stage and in division.

Female 19.5 mm. long (ninety days old). The female gonad may now easily be distinguished from the male gonad by its large oocytes. In some individuals early maturation stages are still quite common, while in others growing oocytes and oogonia are found chiefly. The oocytes in the middle of the gonad are usually larger than those at the anterior and posterior ends, and correlated with this condition the lamellae are also more developed. Figure 60 shows a photograph of a longitudinal section of an ovary of this period. The oogonia are located chiefly near the surface of the lamellae.

The nuclei of the growing oocytes at this stage have taken on a different appearance—one which they retain until practically mature. Previous to this time, a single large nucleolus has been present together with numerous small chromatin particles, but now there are many nucleoli instead of one. The nucleoli usually lie near the nuclear membrane.

Figure 61 is a photograph of a longitudinal section of another ovary of the same age, which shows how the lamellae are formed.

Female 28 mm. long (129 days old). The ovary has now reached a condition which it retains with little change through the remainder of the first year. The oocytes vary in size, due apparently to the difference in the time at which they began maturation. Dividing oogonia as well as early maturation stages are found during the following winter months. Figure 62 shows a transverse section of an ovary of this stage. The space near the ventromedian side is the main part of the ovarian cavity, while the slit through the middle of the section is the space between two adjacent lamellae,

which are cut obliquely. A number of oogonia appear in the section, but they cannot easily be seen with this magnification.

3. Development of the male. Male 9.7 mm. long (fifty-two days old). The male animal is distinguished at this time by the presence of the sperm ducts within the testes. The sperm duct appears first as a small slit in the stroma portion of the gonad. It usually lies near the mesorchium, but sometimes it is found at some distance along the ventral or the median side of the testis. The stroma cells lying around the duct form its epithelial lining. At first these cells are scattered loosely around the lumen, but later they form a distinct layer.

There is no apparent difference between the germ cells of this stage and those of the last, except an increase in number. They usually lie in nests as a result of recent division. Figure 15 is a drawing of a section through a testis of this stage in which three cell nests appear. Surrounding the cell nests and separating them from one another are strands of stroma cells. These stroma cells, as explained before, took their origin in peritoneal cells. The sperm duct is shown as a slit along the ventromedian side.

Male 12.7 mm. long (fifty-nine days old). Figure 63 shows a transverse section of a testis of this period. Near the mesorchium is a blood vessel in which two or three blood cells appear. Near the blood vessel is the main sperm duct with a branch leading out toward a cell nest, which contains a large germ cell and some smaller ones. Other cell nests appear in the section. Branches of the sperm duct, which bud off from the main duct, are found frequently at this time. All branches of the sperm duct will hereafter be called 'secondary ducts' to distinguish them from the primary or main duct.

The large germ cell shown in the figure is of extraordinary size, being one of the largest male germ cells found in any stage. This cell cannot be considered as an oocyte, since it lacks the single large nucleolus and the dense layer of primary yolk which are characteristic of the oocytes of this size. The irregularity of outline of both the cell and the nucleus and also the scattered chromatin indicate that this cell for some reason failed to divide and is degenerating.

Male 14.5 mm. long (seventy-three days old). Germ cells and cell nests are becoming more numerous, mitotic figures being common among both the germ cells and the somatic cells. Figure 64 is a photograph of a cross-section of a testis showing several nests of germ cells. The testis is turned so that the side which is usually lateral lies dorsad. The sperm duct and two blood vessels appear in this section. Blood vessels may usually be distinguished from the sperm ducts at this time by their shape and also by the cell lining. Blood vessels are ordinarily round in cross-section, and the cells lining them are flat and have small nuclei, while the sperm ducts are slits surrounded by thick cells with larger nuclei.

Figure 65 is a photograph of a cross-section of a testis of this age, showing the manner in which most of the smaller secondary ducts are formed. Some of the secondary sperm ducts, particularly the larger ones, form within the mass of stroma near the hilus, but others, especially the smaller ones, form first as dense cords of stroma tissue which grow out from the hilus to the cell nests. In both cases the lumina extend out from the primary duct or from its larger branches.

In one cord, marked *x* in the figure, the cells may be seen arranged in two rows preparatory to the formation of the lumen. The cords shown in the figure are connected with the cell nests at this time, although other stages show that in some cases they are not connected until much later. The connection is apparently made by a disruption of the cell layers surrounding the cell nest and the end of the cord.

Germ cells at this time vary somewhat in size, due chiefly to non-synchronous growth and division. A large nucleolus is always present, and numerous small chromatin particles are found near the nuclear membrane. Along the periphery of the testis is a loose network of stroma cells, which fills up the space not occupied by the germ-cell nests.

Male 16.5 mm. long (ninety days old). Figure 66 is a photograph of a cross-section of a testis of this stage near its caudal end, showing a secondary sperm duct with a lumen leading out to a cell nest. The portion of the secondary duct

next to the primary duct is found in the adjoining section. Two blood vessels appear also in the upper portion of the figure, while in the lower part two secondary ducts, two cell nests, and a dividing germ cell may be seen.

This is the earliest stage in which a secondary sperm duct with a lumen was found leading to a cell nest, although cords of cells which later form branches of the duct were found earlier. The lumina do not extend into the cell nests at this time.

Male 21 mm. long (104 days old). Figure 16 is a drawing illustrating diagrammatically the structure of a testis of this period, as seen in a longitudinal section passing through the sperm duct. The cell nests (*g.c.n.*) are now elongating, preparatory to tubule formation. A blood vessel (*b.v.*) is found near the mesorchium, and near this is the primary sperm duct (*sp.d.*). This is the hilus of the gland, and it usually lies in a dorsomedian position. Secondary sperm ducts lead off from the primary duct and often lie nearly parallel to it for some distance. Many of these branches lead directly to cell nests, while others subdivide into smaller branches before reaching the nests. In many cases cords of stroma cells appear where the smaller branches of the duct have not yet formed. Lumina do not extend into the cell nests at this stage.

Figure 67 is a photograph of a longitudinal section of a testis at this stage, showing the sperm duct, a secondary duct, and numerous cell nests which are cut longitudinally. It is impossible to get a section showing much of the sperm duct because of its convolutions.

Male 25.5 mm. long (129 days old). Occasionally, at this stage, the lumina of the secondary ducts have extended into the cell nests, thus changing the cell nests into tubules. Although the presence of a lumen may be used as a general criterion for distinguishing the tubules from the cell nests, it cannot be made a hard-and-fast one. In stages following this these structures are usually long, slender, and sometimes branched, but often do not contain lumina or the lumina

do not extend to the peripheral ends. On account of the general change in form at this time, however, the cell nests from now on will be called tubules, although in many cases the lumina are not present until early in the second year.

Figure 17 is a drawing of a portion of a tubule showing its connection with the secondary duct. At one side of the tubule is shown a cluster of germ cells budding off. Buds of this nature elongate and form new tubules. A newly formed tubule apparently becomes connected with a secondary duct in one of two ways—either it retains its connection at the base of the old tubule and empties into the same secondary duct or it separates off and becomes connected with an independent branch in the same manner that cell nests first became connected with the secondary ducts. Older stages show that the tubules often remain branched.

Males 152 days to a year old. During the remainder of the first year there is not much change in the testis except a gradual growth. As the testis increases in size, the tubules increase in number and length and become densely crowded together, especially near their inner ends. Although the lumina may be seen clearly in many tubules, in the majority of them at this time the interior appears to be filled with loose strands of tissue. Figure 18 shows a portion of a tubule with branches in an individual nearly a year old (46 mm.). Tubules are usually larger toward the peripheral ends than they are near the hilus, where they are more crowded together. Secondary sperm ducts usually empty into the primary duct at an oblique angle, so that in the cross-section of a testis these branches give the appearance of the primary duct being divided up into several channels.

Division among the germ cells, as indicated by the presence of mitotic figures, occurs until October, then rarely during the winter months.

4. Literature and discussion. Investigators of sex differentiation in teleosts have found two different conditions—one in which there is a tendency toward a juvenile hermaphroditism and another in which the young fish differentiate suddenly and distinctly into males and females.

Grassi ('19) found a juvenile hermaphroditic condition in the eel, and concluded that sex was determined in part by environmental factors, among which were nourishment and temperature. The smaller individuals, he thought, were probably destined to become males, while the medium- and larger-sized ones might become either males or females, depending upon the environment during development. He found many growing oocytes in the testes. D'Ancona ('24) confirmed the work of Grassi.

Mršić ('23) also observed juvenile hermaphroditism in the rainbow trout (Salmo fario) when the young had reached the age of about 131 to 135 days. All gonads at this time contained oocytes and had the appearance of ovaries. A little later in about half of the individuals the oocytes degenerated, and the smaller germ cells increased in number forming the spermatogonia. In the remaining half of the individuals the oocytes continued to grow, producing the definitive ova. Keeping the eggs slightly over time in the body of the mother, he found, resulted in a higher percentage of females.

Essenberg ('23 and '26) found sex reveral in Xiphophorus helleri, which might be taken as evidence of an hermaphroditic condition. In immature individuals he found that the population was 74 per cent females, while in mature fishes it was 75 per cent males. This change, he thought, was brought about by a sex reversal. He also made a study of several mature individuals, which he concluded had changed from the female to the male sex.

An hermaphroditic condition in teleosts is often retained even in the adults. According to MacLeod ('81), this is the rule in Serranus and in the Sparidae. Occasional cases of adult hermaphroditism have been reported also in other species (Smitt, '82; Howes, '91, and others).

In many teleosts in which sex differentiates suddenly, the formation of the oviduct or ovarian cavity and the sperm duct is one of the early indications of sex differentiation. These ducts are not formed in the same manner in the two sexes,

and there is considerable variation in the same sex in different species.

Felix ('06), in reviewing the work of a number of investigators, found that there were four ways in which the teleost oviduct and ovarian cavity were formed: 1) By the gland mass forming in a fold in the dorsal body wall or mesentery, as in the eel (Brock, '78). 2) By the formation of a series of folds on the lateral side of the genital gland, as in the salmon (Brock, '78). 3) By the fusion of the edges of a groove on the surface of the genital fold, as in Belone acus (MacLeod, '81). 4) By the fusion of the edge of the genital fold with the body wall, as in Rhodeus amarus (Jungerson, '89).

The oviduct and the ovarian cavity in Cottus bairdii are formed by the third method mentioned by Felix, namely, by the fusion of the edges of a groove which is usually on the ventral surface of the genital fold. According to MacLeod, the groove was formed in Belone acus at the 54-mm. stage, and the posterior part was formed in advance of the remainder. Jungerson ('89) found in Zoarces that the early ovarian cavity took its origin in a groove, closing first at the anterior end, as it does in Cottus bairdii.

Nussbaum ('80) observed grooves forming around the cell nests in the ovary of the young trout, which he considered as the beginning of the lamellae or later divisions of the ovary. Grooves form in a somewhat similar way in the ovary of Cottus bairdii, except that the cell nests do not play as important a part as in the trout.

According to Felix, the developing testes of teleosts are of two types—the acinus and the radial types. The acinus type (Brock, '78) has small, spherical clusters of germ cells which form lumina, and these become connected with the sperm duct just before spawning time. The radial type (Jungerson, '89) has a strong proliferation of stroma cells around the sperm duct near the hilus, and from here solid cords of stroma cells grow out to the germ cells at the periphery. These cords later form lumina which connect the germ-cell region with

the sperm duct. The testes of Cottus bairdii are clearly of the radial type.

Eigenmann found in Cymatogaster that the sperm duct was first indicated by a solid cord of stroma cells in the dorsal part of the testis, and this later developed into a duct with branches. In Cottus bairdii cords of cells of this nature preceded the formation of the smaller branches, but they were not found where the main duct or larger branches formed.

Jungerson ('89) was able to distinguish the male sex of Gobius by the broader base of the genital fold before he could see any histological difference in the germ cells. In Salmo fario, he thought that in the male the early gonads extended farther caudad than they did in the female, but this is denied by Mršić ('23). Eigenmann ('96) was able to distinguish the sex of Cymatogaster by the form of the gonad before he could see any histological difference in the germ cells. He thought it highly probable, however, that the characteristic form of the gonad was due to some influence exerted by the germ cells.

Cottus bairdii belongs to the type in which sex differentiates suddenly, the sex being indicated by the form of the gonad and a difference in the germ cells at about the same time. There is no indication of hermaphroditism in this form. Before beginning the work, it was thought probable that all fishes might show a juvenile hermaphroditic condition, since this had been found to exist in several widely separated species, and in cyclostomes and amphibians as well. The above study of the period of sex differentiation in Cottus bairdii shows, however, that this condition is not universal in fishes, and it is probable that the cases recorded form exceptions rather than the rule.

C. Oogenesis

1. Observations on oogenesis in Cottus bairdii. *a.* The structure of the mature ovaries. The ovaries of Cottus bairdii are separated anteriorly, but are united at the posterior end where the ovarian cavities join to form the oviduct. The

size of the ovaries varies greatly with the season. Following the spawning season, they are about one-eighth of the length of the body; but as spawning time approaches again, they increase to about one-third the length of the body and to several times their former thickness. The weight of the mature ovaries is about one-third of the weight of the entire body.

The dorsal wall of the ovarian cavity is composed of transverse lamellae which contain various stages of developing eggs. In the adult ovaries the smaller germ cells are usually located in the surface epithelium, while the larger oocytes extend into the interior of the lamellae and are surrounded by a follicular epithelium. At the time of spawning, the eggs burst into the ovarian cavity, leaving the follicles behind, and pass out through the oviduct. Figure 19 shows a ventral view of a pair of ovaries from a 62-mm. female preserved on August 10th. One ovary has had the ventral wall removed to show the arrangement of the lamellae and oocytes in the dorsal wall of the ovarian cavity.

b. The seasonal cycle in the female germ cells. In the young Cottus bairdii, sex differentiation takes place around the second week in June—about seven weeks after the spawning season. In the female, oogonial division and early maturation stages are found until October, but do not appear during the remainder of the first year. Oogonia in a resting condition may be seen within the ovary, however, during the entire year.

A portion of the oocytes which begin development the first year mature when the individual is two years old. An estimate of the number of oocytes of the first generation was made by counting a fraction of them, and this estimate was compared with the number of large oocytes previous to the first spawning season. The results showed that only about 10 per cent mature for the first spawning. The remainder constitute a reserve supply which lies dormant within the ovary. These dormant oocytes vary in size, the largest being about 160 μ. No indication of degeneration or resorption was

observed among them. A difference in the size and appearance of the oocytes which are maturing and the remainder may be noted about June, when the individual is fourteen months old. Figure 68 shows a portion of an ovary preserved on July 4th. Two large oocytes have entered upon the secondary growth phase. The yolk in these has a vacuolated appearance. Figure 69 shows a similar section from an ovary in August.

About the middle of May, when the young female is thirteen months old, the oogonia begin to divide again, and at about the same time some of the cells enter the early maturation phases (fig. 70). This process continues again until October. The cells beginning maturation at this time increase in size and form a part of the reserve supply of oocytes.

After the first breeding season, when the female is about twenty-five months old, oogonial division and early maturation take place again and continue for about the same length of time as during the second year. About June, some of the oocytes from the reserve supply enter the secondary growth phase. Thus each year after the first a portion of the oogonia change to oocytes and increase the reserve supply, and at the same time a portion of the reserve supply enter the secondary growth phase and develop during the year into mature ova. It is seen, therefore, that the eggs of the first spawning season develop in two years, while those of the following seasons take two or more years for their development, depending upon the generation of reserve oocytes from which they are derived.

c. Oogonia. Early in the period of sex differentiation the undivided oogonia do not differ in appearance from the undifferentiated germ cells, but since they are lodged in the ovary and destined to give rise to ova, they may be considered as oogonia. Figure 20 shows one of the large oogonia at the time of sex differentiation. As division proceeds they become slightly smaller and the cell outlines less distinct. Cells of this type are shown in figures 12 and 59. Some of the oogonia change to primary oocytes each year, but there

is always a reserve supply of oogonia present in the ovary. In no case were oogonia observed to develop from the somatic part of the ovary.

d. Primary oocytes. The first indication of maturation is the clumping of the chromatin at one side of the nucleus. This stage, which corresponds to what has been described as synizesis, is one of the early characteristics by which the female sex may be distinguished from the male. Figure 21 shows a cell in this stage. The presence of the nucleolus on the opposite side of the nucleus from the clumped chromatin is also characteristic of this stage. Following synizesis, the chromatin threads scatter through the nucleus, which now grows larger. In two or three weeks, primary yolk formation begins, and the follicle cells become more conspicuous (fig. 22).

Soon after the oocyte begins to grow, several nucleoli appear in the nucleus. These usually lie around the periphery beneath the nuclear membrane (fig. 60). The chromatin threads remain prominent throughout the growth period, but more especially during the early part. Undoubted cases of synapsis have not been observed in the oocyte, although occasionally the threads may have a double appearance.

The oocytes continue to grow slowly during the first year, but all of the first-generation oocytes do not mature at the same time, as stated above. Figure 71 is a photograph of a section of an ovary preserved on May 14th, when the individual was thirteen months old. The oocytes vary in size from 70 μ to 160 μ. A month or so later, some of the oocytes begin to increase in size and exhibit a difference in the appearance of the yolk (figs. 68 and 69). These are the oocytes which will mature for the first spawning. Soon after secondary growth begins, a distinct vitelline membrane forms around the maturing egg (figs. 68 and 69). This membrane is probably derived from the cortical or protoplasmic layer of the egg. At first it is thin and appears homogeneous, but later radial striations appear in it. These striations sometimes appear to continue into the cortical layer beneath. Figure 23 shows the surface membranes of an oocyte on December 22nd, about four months before spawning time.

e. Ripe eggs. The ripe eggs of Cottus bairdii are spherical or slightly oblong in shape, and vary in diameter from 2 to 2.5 mm. The color of the yolk varies from light yellow to orange. All of the eggs from a single individual, however, are of the same size and color. The yolk contains a deposit of small spherical granules on one side which forms a white spot. This substance has a greater specific gravity than the remainder of the yolk and always lies on the lower side. If the egg is inverted, the yolk slowly turns until the white spot again lies below. The eggs, when deposited, are covered with a jelly-like substance which hardens when left in the water for an hour or so, and it is by means of this substance that the eggs become attached to stones and other objects.

f. The number of eggs. The ripening eggs were counted in nine females just before spawning time. The number, as usual, is roughly proportional to the cube of the length. The length of the females and the number of eggs contained in the ovaries are as follows:

Length of female mm.	*Number of eggs*	*Length of female* mm.	*Number of eggs*
51	120	65	243
51	185	66	221
56	273	70	395
62	273	71	341
64	258		

g. The ovary after spawning. Immediately after spawning, the ruptured follicles within the ovary are filled, or partly filled, with cells, derived from the follicular epithelium, forming bodies analogous to corpora lutea (fig. 72). These bodies practically disappear in a few weeks, but traces of them are often found throughout the following year.

2. Literature and discussion. Brock ('78) made a classification of the ovaries of teleosts, based chiefly upon the presence or absence of the oviduct and the lamellae and the arrangement of the lamellae when present. An oviduct is not found in the salmon, but is present in most other forms. Lamellae, when present, are arranged either transversely or longitudinally, and may line either the entire surface of the

ovarian cavity or only one side of it. The ovary of Cottus bairdii corresponds to the Cyprinoid type in Brock's classification. In this type of ovary both lamellae and oviducts are present, the lamellae being arranged transversely on one side of the ovary.

The seasonal cycle of the female germ-cell development in teleosts has been studied by several investigators. Brock ('78) thought that there were two generations of oocytes in the teleost ovary at the same time, except during the period following spawning, but made no attempt to follow the development in any detail. Reighard ('03) found three generations of oocytes in the small-mouthed black bass just before spawning time. Loewe ('03) found in the European shad that oocytes began to develop from oogonia soon after spawning time, and that this transition continued only for a short period. Cunningham ('98) thought that in pelagic teleost eggs the yolk formed only during the year previous to spawning, but he apparently did not examine the ovary for younger stages.

Thompson ('14) found in the ovary of the Pacific halibut that there was a reserve supply of eggs about 0.1 mm. in diameter which numbered about five times as many as were spawned at one time. Miss Clark ('25) found in Leuresthes tenuis that there was a large reserve supply of eggs from 0.08 to 0.23 mm. in diameter in the ovary during the spawning season, and that a small portion of these ripened for each spawning. The condition in Cottus bairdii is comparable to that in the halibut and Leuresthes, except that spawning takes place but once each year in Cottus, while in those forms it occurs several times during the same season.

The observation on Cottus bairdii that the primordial germ cells give rise to ova of successive seasons agrees with the work of a number of investigators on other forms. Loewe ('03) found that in the European shad the primordial germ cells were stored in the ovary and gave rise periodically to the oocytes. Eigenmann ('96) found that in Cymatogaster the germ cells of both sexes had their origin in the primordial

germ cells. Okkelberg ('21) found that in the brook lamprey both male and female germ cells came only from the primordial germ cells. The work of Allen ('09 and '11) on Amia and Lepidosteus and of Beard ('00 and '02) and Woods ('02) on elasmobranchs indicates that in these forms also the primordial germ cells give rise to the definitive male and female sex cells. There is no evidence that the germ cells in Cottus bairdii arise from the germinal epithelium each year, as claimed by Wallace ('03) for Zoarces.

Eigenmann ('90) studied the membranes of a number of teleost eggs and made a classification of the eggs based upon the number and nature of the membrane layers. The zona radiata, or vitelline membrane, was the principal one, and, according to Eigenmann, was derived from the yolk. The egg of Cottus bairdii has only one membrane, the vitelline membrane, besides the follicle, as described by Eigenmann for Notemigonus. In Cottus this membrane apparently is derived from the cortical protoplasm.

The corpora lutea of Zoarces, according to Wallace ('03), are formed from follicle cells as in Cottus bairdii, and bear little resemblance to the complicated corpora lutea of mammals.

The presence of the white spot in the egg of Cottus gobio, according to Surbeck ('00), was noted by Prevost in 1828, and has been noted in Cottus eggs by various observers since, but its real significance is not understood.

D. Spermatogenesis

1. Observations on spermatogenesis in Cottus bairdii. *a.* The structure of the mature testes. The testes of Cottus bairdii vary in size with the season, though the variation is not as great as in the ovary. Following the spawning season, they are about one-tenth the length of the body, but as the next spawning season approaches their dimensions greatly increase. The left testis, when of full size, is usually folded back at the anterior end, on account of the stomach obstructing the cavity. A testis is commonly triangular in cross-

section, and is bounded by the gut, the dorsal wall of the coelom, and the lateral body wall. The sperm duct lies on the median or dorsomedian side, near the attachment of the mesorchium (fig. 74). The two sperm ducts unite at the posterior ends of the testes, and the common duct leads out through the genital papilla. The histological structure of the testes varies with the time of year, as described below.

b. The seasonal cycle in the male germ cells. In the study of sex differentiation, the history of the male germ cells was followed to the end of the first year. At this time the sperm duct and its branches are formed and also the tubules which empty into the duct. Often no lumina are yet formed in the tubules at the end of the first year. This is particularly true of the peripheral portion of the testis.

In May, when the individual is thirteen months old, the germ cells begin to divide again after a rest during the winter, and division continues until September, when maturation begins. Also, lumina continue to form in the tubules and are practically complete by September. During the summer, cell division takes place quite rapidly, and numerous cysts form within the walls of the tubules. These cysts are formed from a single cell by successive divisions within a thin covering of follicle cells. The divisions occur in all the cells of a cyst at practically the same time. Except when the cells are dividing, the cysts at this time are not easily distinguished, since the walls are very thin, but close observation will detect their presence.

With the beginning of maturation the testes take on a very different appearance. The cysts show very distinctly now, and all the cells of a cyst are in practically the same stage, but various stages appear in different cysts (fig. 73). Maturation continues through the winter months until spawning time in the spring. Figure 74 shows a cross-section of a testis in December. The tubules appear dark, on account of the presence of the spermatid masses, which will be discussed later. The radial arrangement of the tubules with regard to the sperm duct may be seen here. Following spawning,

the testes and ducts always contain some sperm, but by the end of a month these have nearly all disappeared.

An examination of the testes during the maturation period discloses a number of germ cells lying dormant along the sides of the tubules (fig. 73). After the spawning season and the disappearance of the old sperm, these germ cells begin to divide and fill up the tubules as the first set of cells did. Figure 75 shows a photograph of a section of testis preserved on June 18th, about two months after the spawning season. The germ cells may be seen here within the old tubules. The walls of the old cysts are also present and to some extent help to form walls for the cysts of the following season. Nuclei of the follicle cells may be seen in the figure. The division of the germ cells begins in May and continues until maturation begins in September.

c. Spermatogonia. Figure 24 shows a spermatogonium from a testis preserved on August 27th, a short time before the beginning of maturation. A nucleolus is present at this time and the chromatin lies scattered through the nucleus. Division figures are found frequently. A polar view of an equatorial plate is shown in figure 25. The chromosomes appear round or oval, and are a little less than 1 μ in length. Some of the chromosomes in the figure lie against each other, but this does not represent division.

d. Primary spermatocytes. At the beginning of this stage the nucleolus disappears and the chromatin collects at one side of the nucleus in synizesis (fig. 26). This stage is similar to the corresponding one in the oocyte, except that the nucleolus does not disappear in the oocyte. Following synizesis, the chromatin disperses throughout the nucleus, forming a network of chromatin threads (fig. 27). The threads later collect beneath the nuclear membrane and form the haploid number of chromatin bodies representing the tetrad stage (fig. 28). Nuclei at this time are about 6 μ in diameter.

e. First meiotic division. Figure 29 shows an equatorial plate of a first meiotic division. The chromatin bodies usually appear round or oval, and do not ordinarily show their

double nature. There are apparently half as many chromatin bodies, however, as in the spermatogonia, as indicated in counts considered later. No heterochromosome could be distinguished at any stage.

f. Secondary spermatocytes. Figure 30 shows a secondary spermatocyte. These are smaller than the primary spermatocytes and are found less frequently on account of their shorter duration. The chromatin in some cases appears to be diffused throughout the nucleus, while in others it remains in the form of chromosomes, as in the figure.

g. Second meiotic division. In this division the chromosomes are usually clumped together. Division stages are often found scattered among the secondary spermatocytes. Figure 31 shows an equatorial plate of a second meiotic division. No chromosome counts were possible at this stage.

h. Spermatids. After the second meiotic division the chromatin scatters through the nucleus of the spermatid, giving it the appearance of a resting nucleus (fig. 32). The chromatin then gathers at one side, forming a cup-shaped hemisphere along the nuclear membrane. These hemispheres appear as half-circles or crescents in cross-section (fig. 33). Outside of the nuclear membrane near the middle of the chromatin mass is a black-staining body which appears to be the centrosome. After this the chromatin mass becomes more regular in contour and assumes the general shape of a sperm head. Viewed from the flat side, these appear oval, and in the middle one or two areas stain less deeply than the remainder (fig. 34). In profile they have the shape shown in figure 35.

i. Sperm. The ripe sperm has a disc-shaped head which appears slightly oval when seen from the flat side (fig. 36). A portion of the middle does not stain as deeply as the remainder, and this often makes the head appear ring-shaped, or horseshoe-shaped. From the edge the head appears convex on one side and slightly concave on the other (fig. 37). There is also a distinct middle piece and a long tail. The head measures about 2 μ in length. The middle-piece varies in length from 2 μ to 4 μ.

j. Chromosome counts. Chromosome counts could not be made with any high degree of accuracy on account of the minute size of the chromosomes. However, a number of counts were attempted and the averages taken which gave approximate numbers. An average of twenty-seven counts in spermatogonial divisions gave 35.2 for the diploid number, while an average of fifty counts just preceding the first meiotic division gave 17.8 for the haploid number. The number is more likely to be too low than too high, for in some cases undoubtedly some of the chromosomes were missing in the sections counted. Figure 29 shows one of the clearest equatorial plates of the first meiotic division, and nineteen chromatin bodies are rather definitely shown. An estimate of the chromosome number may be placed at thirty-six or thirty-eight, but this is meant to be only approximate.

2. Literature and discussion. Brock ('78) studied the testes of about thirty species of teleosts and divided them into two groups on the basis of general structure. The Acanthopterygian type of testis had tubules radiating from the sperm duct to the periphery. A few of these tubules emptied directly into the sperm duct, but usually from two to six of them emptied into a common duct, which then emptied into the sperm duct. The Cyprinoid type had branched tubules, which anastomosed and formed a network, so that the real structure could not easily be determined. The testis of Cottus bairdii is plainly of the Acanthopterygian type.

Turner ('19) described the testis of the perch as having a connective-tissue core and strands of connective tissue radiating out to the periphery, dividing the testis into lobules. These lobules were further divided into cysts during the maturation period. He thought also that there was a cord of germ cells outside of the testis from which the testis was periodically supplied. Van Oordt ('24) described the testis of the stickleback, Gasterosteus pungitius, as having numerous sperm canals ending blindly at the periphery of the testis and uniting in the center with the sperm duct. The canals were divided up into cysts which contained cells, all in

the same stage of development. The tubules in Cottus bairdii evidently correspond to the 'lobes' in the testis of the perch as described by Turner and the 'canals' of the stickleback as described by van Oordt. The cysts within the tubules are also similar to those described by Turner and van Oordt, but no cord of germ cells was found outside of the testis as Turner described for the perch.

Geiser ('24), in Gambusia holbrooki, recognized leptotene, synaptene, and pachytene stages in the primary spermatocyte, but found no synizesis. Tetrads, he thought, were of the open-ring type. In Cottus bairdii the synizesis stage is quite outstanding, but the stages which Geiser found could not be recognized. The hemispherical mass of chromatin in the spermatid, however, is similar to a corresponding stage which he described for Gambusia.

Ballowitz ('90 and '15 a) described the sperm of a number of teleosts. The parts of the sperm noted were the head, middle-piece, tail, fringe of the tail, and the end-piece. In some species the fringe and end-piece were lacking. The periphery of the head always stained more deeply than the central part. In some species the axis thread extended into the head and ended in a small spherule. In another article ('15 b) he described more fully the herring sperm in a reply to Retzius ('10), who said that the herring sperm was spherical. Ballowitz contended that the spherical form was due to swelling before fixation. The herring sperm was about 2 μ in length and the pike sperm 2.2 μ. Waldeyer ('01–'03) says that teleost sperm are the smallest of all sperm. The sperm of Cottus bairdii is about the same length as the herring sperm. The head is shaped much like that of Zoarces, according to Ballowitz's figures, but the middle-piece is longer. The head contains the light spot which he described for teleost sperm, but no spherule was noted and no fringe was found on the tail.

E. Spermatid masses

General characteristics. Within the tubules of the ripening testis of Cottus bairdii there are formed some peculiar bodies

which deserve special consideration. The nature of these bodies has not been fully ascertained, but in the present description they will be referred to as 'spermatid masses.'

These bodies appear in the cysts when the cells are in the spermatid stage and persist until the time of spawning. In early stages they vary considerably in size, but later in the season they become more nearly uniform as a result of an increase in the size of the smaller masses. The largest of the masses measure about 10 μ in diameter. Figure 76 is a photograph of a testis on the first of February. The smaller bodies are spermatids and spermatozoa, and the larger ones the spermatid masses. Figure 77 shows a testis on the first of April, three weeks before spawning time, in which the smaller bodies are spermatozoa and the larger ones, the spermatid masses. These masses often are so abundant that they crowd the tubules, leaving apparently very little room for the spermatozoa. Fresh milt at spawning time also contains these masses, which lie inert among the wriggling sperm.

The spermatid masses appear different in different preparations. With Bouin's fixation and iron-hematoxylin they stain black, while with Flemming's solution and iron-hematoxylin they become gray and somewhat transparent. Flemming's fixation gives the better preparation for study, since after it one can see to some extent the nature of the internal structure.

In early stages, particularly, the masses appear to be made up of smaller bodies, but in older stages they usually have a homogeneous appearance. Sometimes there appears to be a thin membrane on the outside which takes a cytoplasmic stain. Figures 38 to 40 show drawings of some of these masses which appear to be made up of smaller bodies. Figures 41 to 47 show similar ones in outline. Figures 48 and 49 show outlines of masses which are more uniform in contour, such as occur in the more advanced stages. These are all camera-lucida drawings with a magnification of 1875 diameters. In fresh material the masses are white or cream-colored and

appear to have a homogeneous interior. If pressed down by the cover-glass they flatten out and burst. When crushed, they sometimes run together like drops of mercury. There is no evidence in the crushed masses that they contain developing structures of any kind.

Tests were made with scharlach R and osmic acid on fresh material to see if these masses might not be fat-bodies, but the results were negative in both cases.

Origin. The spermatid masses are apparently formed by the fusion of spermatids. The evidences which indicate this origin are the following: 1) They are always formed in the presence of spermatids. 2) When these masses are formed, the number of spermatids decreases. 3) They often have shapes indicating that they have been formed from smaller bodies, such as the spermatids. The size varies greatly, also, during early stages, as if they were formed by degrees. 4) They stain like the chromatin of the spermatids.

Figure 50 shows two spermatids with the convex sides of the chromatin masses together as if they were uniting. This condition is found quite frequently among the spermatids, in some cases several of them occurring in one cyst. This may be the first step in the formation of the spermatid masses.

DISCUSSION

This is the first time, to my knowledge, that bodies of this nature have been described. They do not correspond with the sperm packets which Courrier ('22) and van Oordt ('24) found in the stickleback, for in these the sperm with tails were plainly visible. Neither do they correspond with the spermatozeugmata of the viviparous poeciliids described by different works. The spermatozeugmata described by Henn ('16) were 122 μ in length in one species and 220 μ in another, while the masses in Cottus bairdii do not ordinarily exceed 10 μ in diameter.

Although the true nature of the spermatid masses has not been ascertained, two explanations may be suggested.

1. They might be packets of ripening sperm. They occur just at the time one might expect such packets to form if they were present, and apparently they are formed by the uniting of spermatids. The facts that all spermatids do not enter the masses, however, and that no sperm have been discovered within them, leave little support for this theory.

2. Another suggestion is that they are formed by the fusion of the chromatin of aberrant spermatids, which do not develop further. The staining reaction and the homogeneous appearance of later stages lend considerable support to this view, which has tentatively been adopted in this study. The objection may be raised, however, that such cells would likely degenerate and be resorbed, while these masses remain intact.

These masses will be the subject of a more thorough investigation in the future, when perhaps their structure and function can be more definitely ascertained.

SUMMARY

1. The spawning of Cottus bairdii in the vicinity of Ann Arbor, Michigan, occurred around April 20th, in the year when the most intensive study was made. The incubation period during the same year was approximately thirty-six days. Eggs kept in the laboratory at a temperature of about 15°C. hatched in twenty days.

2. The manner of spawning was observed in the laboratory.

3. The primordial germ cells are derived from giant cells, which are large, undifferentiated cells of entodermal origin. Giant cells were first found in a 1.64-mm. embryo before the gut was formed. Later they are found along the ventral and lateral margins of the gut, and from here a portion of them pass into the undivided lateral mesoderm. When the gut shifts ventrad, some of the giant cells in the lateral mesoderm move to a position dorsal to the gut, become surrounded by peritoneal cells, and first become distinctly recognizable as germ cells. From here they move to a median position and then laterad to the germ-gland anlagen.

4. The number of the primordial germ cells varies greatly in different individuals, counts ranging from twelve to eighty. The tendency toward a high or a low number is apparently characteristic of all the embryos of the same cluster of eggs.

5. Secondary division among the germ cells takes place first about hatching time.

6. Sex could not be distinguished at forty-eight days, but was well established at fifty-two days. The female sex is indicated by early maturation stages of the germ cells and by a groove on the ventral surface of the ovary, which is the beginning of the ovarian cavity. The male sex is indicated by the presence of the sperm duct within the gonad. Sex differentiates suddenly and no tendency toward hermaphroditism is apparent.

7. The definitive sex cells in both sexes have their origin only in the primordial germ cells. No transition from somatic cells to germ cells was found at any stage.

8. Oogonial division and early maturation stages of the oocytes are found first at the time of sex differentiation, and then continuously through the remainder of the summer. When the female is about fourteen months old, a small portion of the first generation of oocytes begins to ripen for the first spawning, which takes place when the female is two years old. The remainder of them lie dormant within the ovary. During the second and each succeeding summer, more oocytes are formed and added to the reserve supply. Likewise, during each summer following the spawning season, a portion of the reserve supply of oocytes begins to ripen for the next spawning season. Oocytes are formed only from oogonia which are present within the ovary.

9. Maturation in the male begins in September preceding the spawning and continues until spawning time. Germ cells which lie dormant within the cysts during maturation divide during the following summer and give rise to the next generation of male germ cells. The diploid number of chromosomes is about thirty-six or thirty-eight.

10. During the maturation of the male germ cells, 'spermatid masses' appear in the cysts at the spermatid stage. These bodies are about 10 μ in diameter, are inert, and stain like chromatin. They are probably formed by a fusion of spermatids.

BIBLIOGRAPHY

Allen, B. M. 1907 A statistical study of the sex-cells of Chrysemys marginata. Anat. Anz., Bd. 30.

———— 1909 The origin of the sex-cells of Amia and Lepidosteus. Anat. Rec., vol. 3.

———— 1911 The origin of the sex-cells of Amia and Lepidosteus. Jour. Morph., vol. 22.

Bachmann, Freda M. 1914 The migration of the germ cells in Ameiurus nebulosus. Biol. Bull., vol. 26.

Ballowitz, E. 1890 Untersuchungen über die Struktur der Spermatozoen. III. Fische, Amphibien und Reptilien. Arch. f. mikr. Anat., Bd. 36.

———— 1915 a Über die Samenkörper der Forellen. Arch. f. Zellforsch., Bd. 14.

———— 1915 b Zur Kenntnis der Spermien des Herings. Arch. f. Zellforsch., Bd. 14.

Beard, J. 1900 The morphological continuity of the germ-cells in Raja batis. Anat. Anz., Bd. 18.

———— 1902 The germ-cells of Pristiurus. Anat. Anz., Bd. 21.

Berenberg-Gossler, H. von 1914 Über Herkunft und Wesen der sogenannten primären Urgeschlechtszellen der Amnioten. Anat. Anz., Bd. 47.

Böhi, U. 1904 Beiträge zur Entwicklungsgeschichte der Leibeshöhle und der ersten Genitalanlage bei den Salmoniden. Morph. Jahrb., Bd. 32.

Brock, J. 1878 Beiträge zur Anatomie und Histologie der Geschlechtsorgane der Knochenfische. Morph. Jahrb., Bd. 4.

———— 1881 Untersuchungen über die Geschlechtsorgane einiger Muraenoiden. Mitt. zool. Stat. Neapel, Bd. 2.

Clark, Frances 1925 The life history of Leuresthes tenuis, an atherine fish with tide controlled spawning habits. Fish Bull. no. 10, Calif. Fish and Game Com.

Courrier, R. 1922 Étude préliminaire du déterminisme des caractères sexuels secondaires chez les poissons. Arch. d'Anat., d'Histol., et d'Embryol., T. 2.

Cunningham, J. T. 1898 On the histology of the ovarian ova of certain marine fishes. Quart. Jour. Micr. Sc., vol. 40.

D'Ancona, U. 1924 Sulla determinazione del sesso nell anguilla. R. Comit. Talass. Ital. Mem. 110.

Dodds, G. 1910 Segregation of the germ-cells of the teleost, Lophius. Jour. Morph., vol. 21.

Eigenmann, C. H. 1890 On the egg membranes and micropyle of some osseous fishes. Bull. Mus. Comp. Zool. Harvard, vol. 19.

———— 1891 On the precocious segregation of the sex-cells in Cymatogaster aggregatus. Jour. Morph., vol. 5.

EIGENMANN, C. H. 1896 Sex-differentiation in the viviparous teleost Cymatogaster. Arch. Entw.-Mech., Bd. 4.

ESSENBERG, J. M. 1923 Sex-differentiation in the viviparous teleost Xiphophorus helleri. Biol. Bull., vol. 45.

——— 1926 Complete sex-reversal in the viviparous teleost Xiphophorus helleri. Biol. Bull., vol. 51.

FATIO, V. 1882 Faune des vértébres de la Suisse, T. 4. Genève et Bale.

FEDEROW, V. 1907 Ueber die Wanderung der Genitalzellen bei Salmo fario. Anat. Anz., Bd. 31.

FELIX, W. 1906 Geschlechtsdrüsen der Teleostier. Handbuch der Entwickelungslehre der Wirbeltiere (Hertwig's), Bd. 3.

FRAENKEL, F. 1913 Haltung und Zucht der Groppe (Cottus gobio). Bl. f. Aquarien- u. Terrarienkunde, Jahrg. 24.

GAGE, S. H. 1878 Notes on the Cayuga Lake star gazer. Cornell Review, Nov., 1878.

GATENBY, J. B. 1916 The transition of peritoneal epithelial cells into germ cells in some Amphibia Anura, especially in Rana temporaria. Quart. Jour. Micr. Sc., vol. 61.

GEISER, S. W. 1924 Sex-ratios and spermatogenesis in the top-minnow, Gambusia holbrooki. Biol. Bull., vol. 47.

GRASSI, B. 1919 Nuove ricerche sulla storia naturale dell'Anguilla. R. Com. Talass. Ital., Mem. 67.

HENN, A. W. 1916 On various South American Poeciliid fishes. Ann. Carnegie Mus., vol. 10.

HERTWIG, R. 1905 Ueber das Problem der sexuellen Differenzierung. Verh. deutsch. zool. Gesellsch., Vers. 15.

HOFFMAN, C. K. 1886 Zur Entwicklungsgeschichte der Urogenitalorgane bei den Anamnia. Zeitschr. wiss. Zool., Bd. 44.

HOWES, G. B. 1891 On some hermaphroditic genitalia of the codfish (Gadus morrhua), with remarks upon the morphology and phylogeny of the vertebrate reproductive system. Jour. Linn. Soc., vol. 23.

JUNGERSON, H. F. 1889 Beiträge zur Kenntnis der Entwickelung der Geschlechtsorgane bei den Knochenfischen. Arb. zool.-zootom. Inst. Würzburg, Bd. 9.

LOEWE, FR. 1903 Über Neu- und Rückbildung im Ovarium vom Maifisch. Arch. mikr. Anat., Bd. 63.

MACLEOD, J. 1881 Recherches sur la structure et la développement de l'appareil reproducteur femelle des Téléostéens. Arch. de Biol., T. 2.

MRŠIĆ, W. 1923 Die Spätbefruchtung und deren Einfluss auf Entwicklung und Geschlechtsbildung, experimentell nachgeprüft an der Regenbogenforelle. Arch. mikr. Anat., Bd. 98.

NUSSBAUM, M. 1880 Zur Differenzierung des Geschlechts im Thierreich. Arch. mikr. Anat., Bd. 18.

OKKELBERG, P. O. 1921 The early history of the germ cells in the brook lamprey, Entosphenus wilderi (Gage), up to and including the period of sex differentiation. Jour. Morph., vol. 35.

OORDT, G. J. VAN 1924 Die Veränderungen des Hodens während des Auftretens der sekundären Geschlechtsmerkmale bei Fischen. Arch. mikr. Anat., Bd. 102.

REIGHARD, J. 1903 The breeding habits, development, and propagation of the black bass. Bull. Mich. Fish Com., no. 7.

REINHARD, L. 1924 Die Entwicklung des Parablasts und seine Bedeutung bei Teleostiern nebst der Frage über die Entstehung der Urgeschlechtszellen. Arch. mikr. Anat., Bd. 103.

RETZIUS, G. 1905 Die Spermien der Leptokardier, Teleostier und Ganoiden. Biol. Untersuchen, N.F., Bd. 12.

RICHARDS, A., AND THOMPSON, J. 1921 The migration of the primary sex-cells of Fundulus heteroclitus. Biol. Bull., vol. 40.

RÜCKERT, J. 1888 Ueber die Entstehung der Excretionsorgane bei Selachiern. Arch. Anat. Phys., Anat. Abt., Jahrg. 1888.

SCHREITMÜLLER, W. 1925 Zur Biologie der Groppe (Cottus gobio L). Bl. f. Aquarien- u. Terrarienkunde, Jahrg., 36.

SINK, E. 1912 The origin of the germ cells in the toadfish (Opsanus tau). Mich. Acad. Sci. Rept., no. 14.

SMITH, B. G. 1922 Notes on the nesting habits of Cottus. Papers of Mich. Acad. Sci. Arts and Letters, vol. 2.

SMITT, F. A. 1882 Description d'un hareng hermaphrodite. Arch. de Biol., T. 3.

———— 1892 A history of Scandinavian fishes. Stockholm.

SURBECK, G. 1900 a Ein Copulationsorgan bei Cottus gobio. Zool. Anz., Bd. 23.

———— 1900 b Das 'Copulationsorgan' von Cottus gobio. Zool. Anz., Bd. 23.

THOMPSON, W. F. 1914 A preliminary report on the life history of the halibut. Rept. British Columbia Fish. Com., 1914.

TURNER, C. 1919 The seasonal cycle in the spermary of the perch. Jour. Morph., vol. 32.

WALDEYER, W. 1870 Eierstock und Ei. Leipzig.

———— 1901–1903 Die Geschlechtszellen. Handbuch der Entwickelungslehre der Wirbeltiere (Hertwig's), Bd. 1.

WALLACE, W. 1903 Observations on ovarian ova and follicles in certain teleostean and elasmobranch fishes. Quart. Jour. Micr. Sci., vol. 47.

WILSON, H. V. 1889 The embryology of the sea bass (Serranus atrarius). Bull. U. S. Fish Com., vol. 9.

WOODS, F. A. 1902 Origin and migration of the germ-cells in Acanthias. Am. Jour. Anat., vol. 1.

WYHE, J. W. VAN 1889 Ueber die Mesodermsegmente des Rumpfes und die Entwicklung des Exkretionsystems bei Selachiern. Arch. mikr. Anat., Bd. 33.

PLATES

ABBREVIATIONS

an., anus
ana., anaphase of oogonial division
b.c., blood cell
br., branch
b.v., blood vessel
c.l., cortical layer
c.lu., corpus luteum
c.str.c., cord of stroma cells
cy., cyst
d.a., dorsal aorta
d.g.c., dividing germ cell
d.oog., dividing oogonium
end.c., endothelial cell
ent., entoderm
f., follicle
f.c., follicle cell
fi., fissure
g.c., germ cell
g.c.n., germ-cell nest
g.c.r., germ-cell region
gi.c., giant cell
gi.c.r., giant-cell region
g.r., genital ridge
gr.ooc., growing oocyte
lam., lamella
l.oog., large oogonium
lum., lumen
mat.ooc., maturing oocyte
mes., mesoderm
mesen., mesentery
mesor., mesorchium
mesov., mesovarium
n.c., neural cord
oog., oogonium
ov.c., ovarian cavity
ov.g., oviducal groove
ovid., oviduct
p., periblast
per.c., peritoneal cell
p.n., periblast nucleus
res.ooc., reserve oocyte
s., spermatid
s.m., spermatid mass
s.oog., small oogonium
sp., sperm
sp.d., sperm duct
sp.d.^{2}, secondary sperm duct
spg., spermatogonium
str.c., stroma cell
syn., synizesis stage of maturation
tel., telophase stage of dividing oogonium
tub., tubule
v.d., vas deferens
v.m., vitelline membrane
w.d., wolffian duct
y., yolk

PLATE 1

EXPLANATION OF FIGURES

1 Section through the middle of a 1.05-mm. embryo. × 175.

2 Section through a 1.64-mm. embryo just anterior of Kupffer's vesicle. × 380.

3 A ventrolateral portion of a section through a 1.91-mm. embryo. × 380.

4 Lateral portion of a section through a 1.98-mm. embryo. × 380.

5 Embryo 2.04 mm. long. The black area represents the giant-cell region. Lines *ab* and *cd* are the planes of figures 52 and 51. × 33.

6 Section through the germ-gland region of an embryo 3.21 mm. long. × 345.

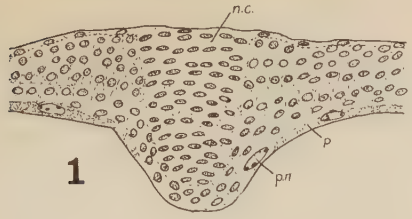
7 Embryo 4.25 mm. long. The black area represents the germ-cell region. Line *ab* is the plane of the section in figure 55. × 13.

8 Section through the genital ridges of a 4.7-mm. embryo. × 345.

9 Section through the genital folds of a 6.5-mm. individual at hatching time. × 345.

10 Section through the genital gland of a 7.3-mm. individual. × 750.

11 Section through the genital gland of an 8.9-mm. individual, forty-eight days old, just before sex differentiation. × 380.



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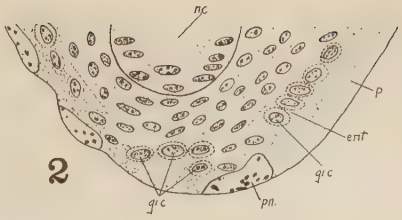

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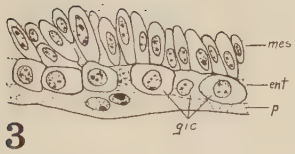

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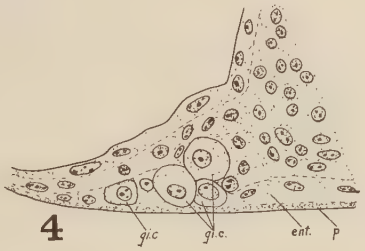

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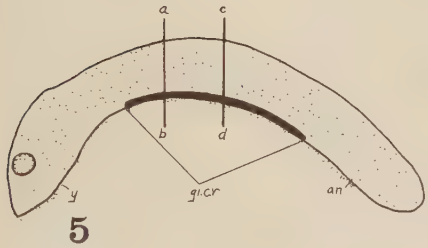

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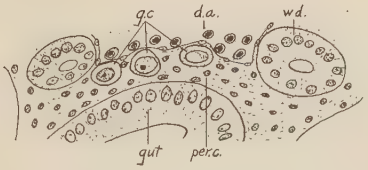

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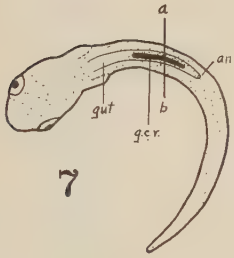

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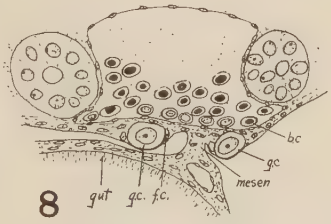

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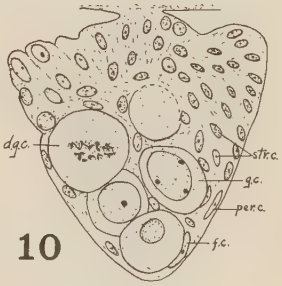

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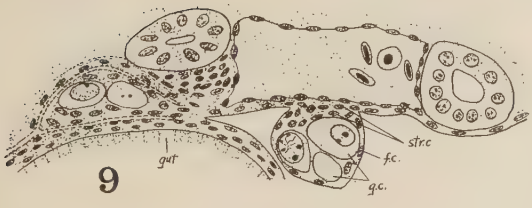

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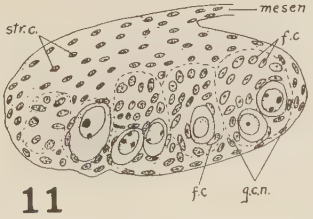

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PLATE 2

EXPLANATION OF FIGURES

12 a, b, and c, sections through the anterior, middle, and posterior portions of the ovaries of a fifty-two-day female. × 175.

13 a, b, and c, outline drawings of sections through the anterior, middle, and posterior portions of the ovaries of a fifty-two-day female.

14 Outline drawing of a section through the middle of the ovaries of a fifty-nine-day female. × 175.

15 Section through the testis of a fifty-two-day male. × 380.

16 Diagram of a longitudinal section through the testis of a 104-day male.

17 Longitudinal section of a tubule and its connection with a secondary duct. A branch to the main tubule is forming. One hundred and twenty-nine-day male. × 750.

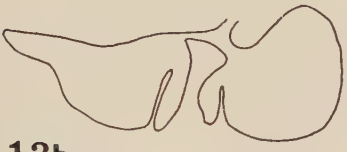
18 Longitudinal section of a tubule and branches from the testis of a year-old male. × 345.

12a

12b

12c

13a



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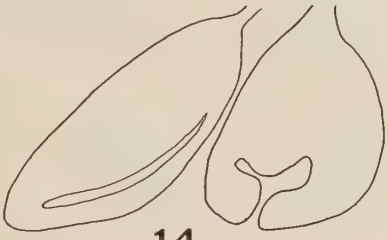

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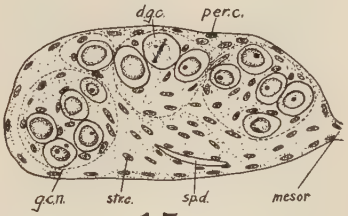

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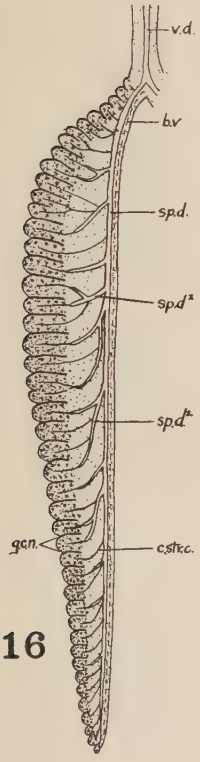

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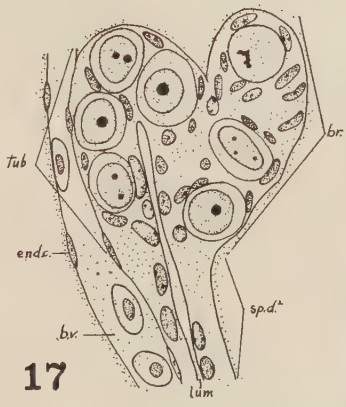

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18

PLATE 3

EXPLANATION OF FIGURES

19 Ventral view of the ovaries of a 62-mm. female on August 10th. × 6.

20 Oogonium from a female at early sex differentiation time (fifty-two-day stage). × 1875.

21 Oocyte in synizesis stage, from the same individual. × 1875.

22 A growing oocyte from a seventy-three-day female. × 750.

23 The surface layers of a ripening oocyte, four months before spawning. × 1875.

24 Spermatogonium from a 50-mm. male on August 27th. × 1875.

25 Equatorial plate of a spermatogonial division. × 1875.

26 Primary spermatocyte in the synizesis stage. × 1875.

27 Primary spermatocyte following synizesis. × 1875.

28 Primary spermatocyte with the haploid number of chromatin bodies lying near the nuclear membrane. × 1875.

29 First meiotic division plate. × 1875.

30 Secondary spermatocyte. × 1875.

31 Second meiotic division plate. × 1875.

32 Spermatid in resting stage. × 1875.

33 Hemispherical mass of chromatin in the spermatid following the resting stage. × 1875.

34 Spermatid in which the hemispherical mass has become disc-shaped. Seen from the flat side. × 1875.

35 Same kind of spermatid seen from the edge. × 1875.

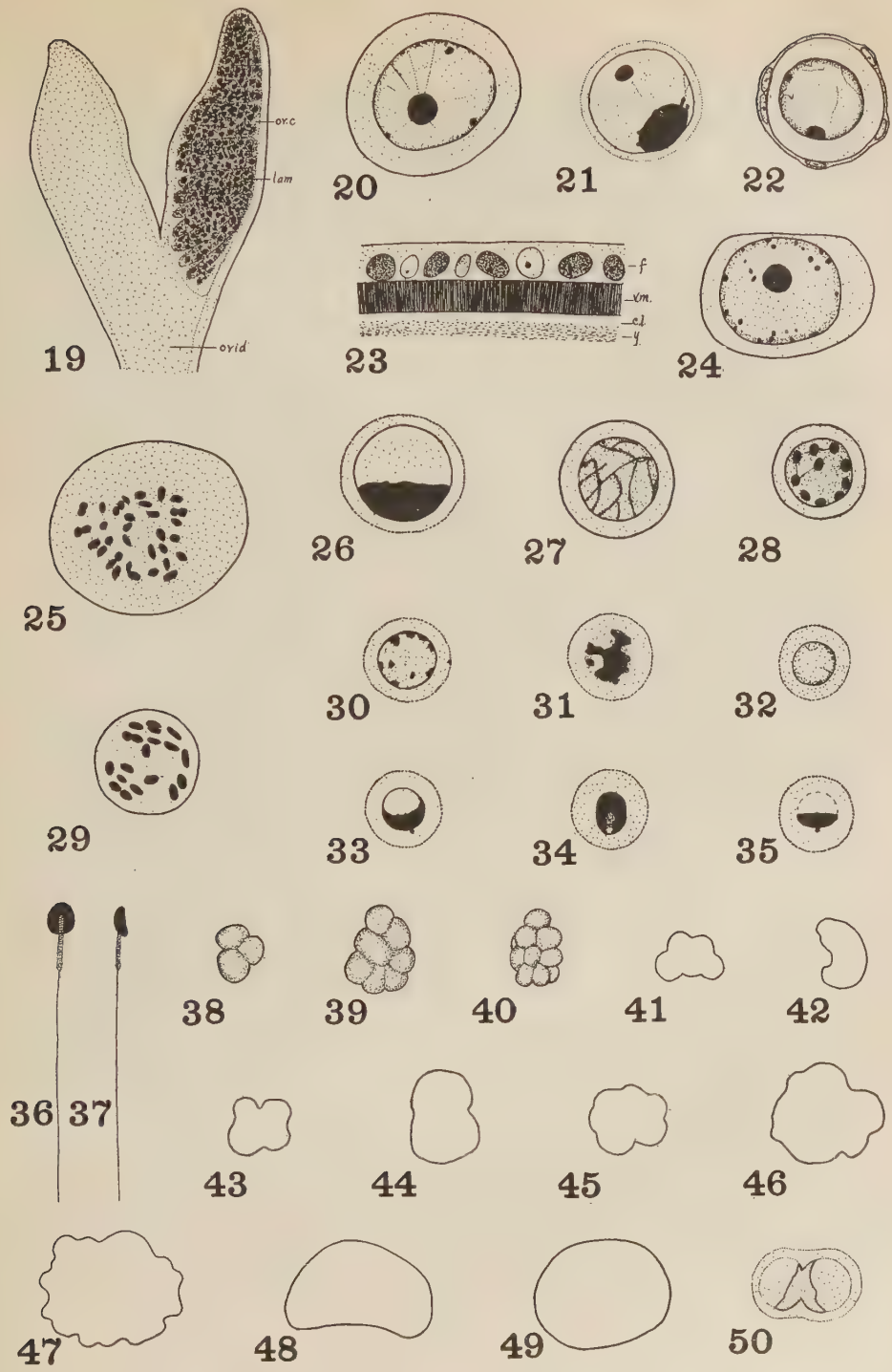
36 Sperm seen from the flat side. × 1875.

37 Sperm seen from the edge. × 1875.

38 to 40 Spermatid masses which show the component parts, after Flemming's fixation. × 1875.

41 to 49 Spermatid masses shown in outline. × 1875.

50 Two spermatids in which the chromatin discs appear to be fusing. × 1875.



19 20 21 22 23 24 25 26 27 28 29 30 31 32 33 34 35 36 37 38 39 40 41 42 43 44 45 46 47 48 49 50

PLATE 4

EXPLANATION OF FIGURES

51 Section of a 2.04-mm. embryo at line *cd* in figure 5. × 395.

52 Section from the same embryo at line *ab* of figure 5. × 395.

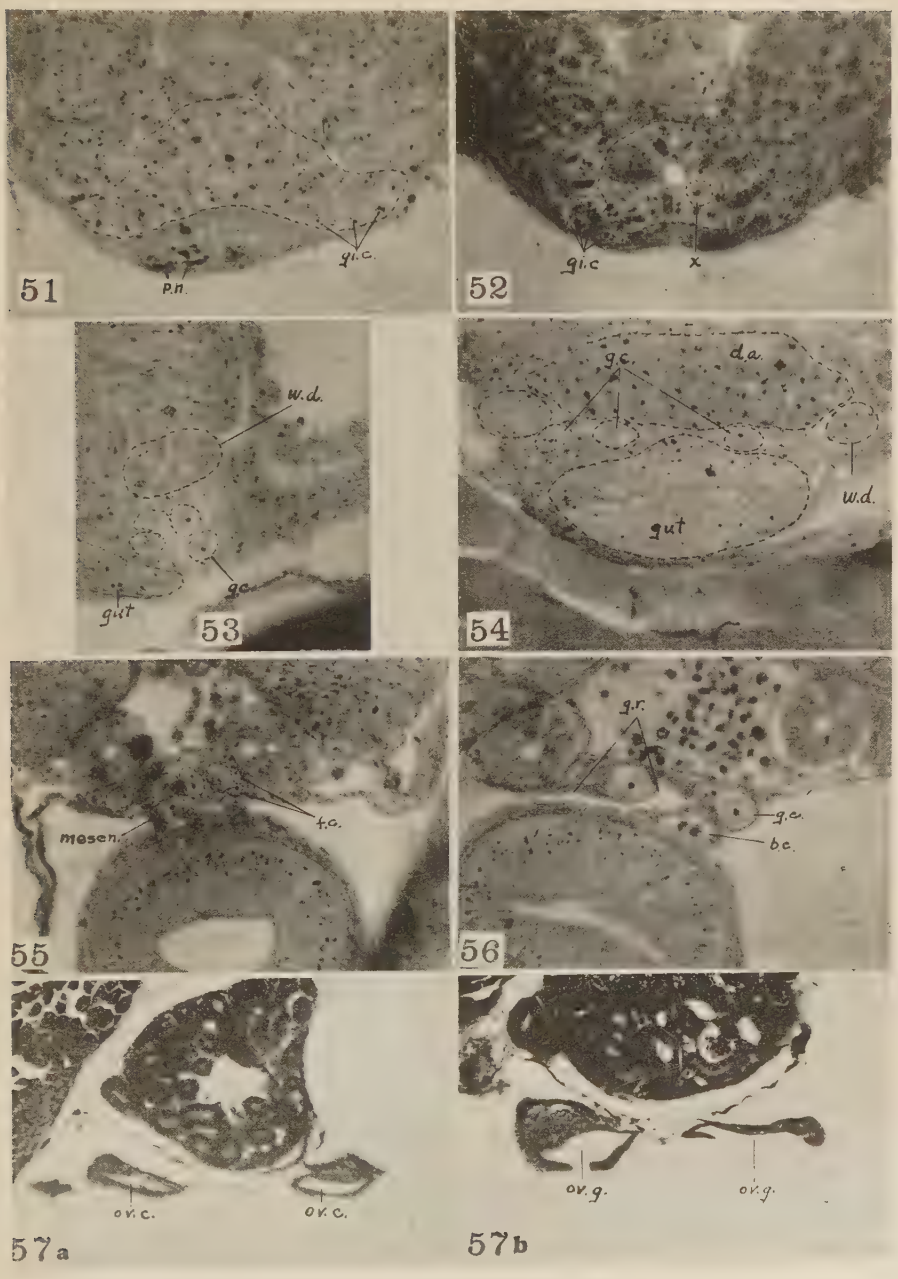
53 Lateral portion of a section from a 2.36-mm. embryo. × 395.

54 Section of a 2.77-mm. embryo. × 395.

55 Section of a 4.25-mm. embryo at line *ab* in figure 7. × 395.

56 Section of a 5.3-mm. embryo. × 395.

57 a, b, c, and d, sections through the ovaries of a fifty-nine-day female. Figure a is through the anterior end; b is through the middle; c is through the posterior portion where the cavities are separate, and d is where the cavities are united to form the oviduct. × 85. (Figs. 57, c and d, on p. 491.)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gl.c.
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g.c.
bc.
57a
ov.c.
ov.c.
57b
ov.g.
ov.g.

PLATE 5

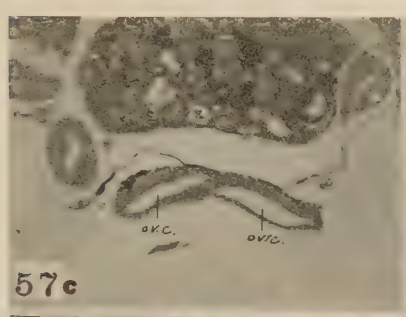
EXPLANATION OF FIGURES

58 Longitudinal section through the ovary of a seventy-three-day female, showing the formation of the lamellae. × 185.

59 Longitudinal section through the ovary of another seventy-three-day female. × 395.

60 Longitudinal section through the ovary of a ninety-day female. × 185.

61 Longitudinal section through the ovary of another ninety-day female. × 85.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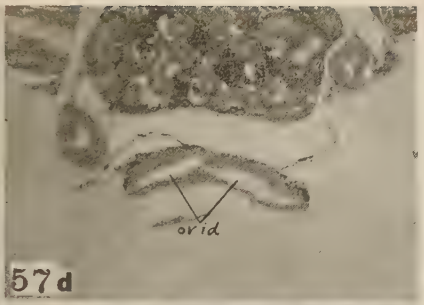
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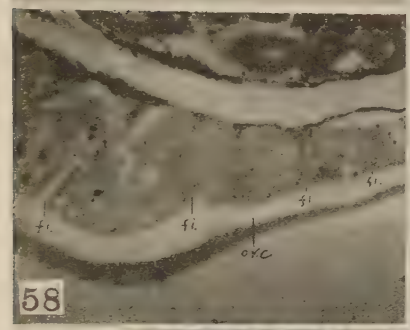
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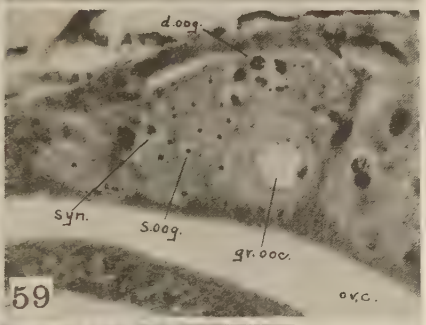
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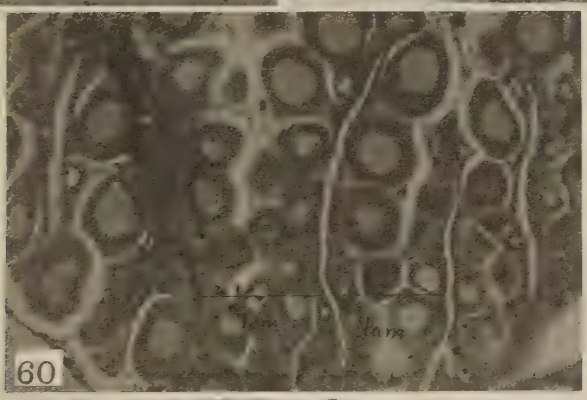
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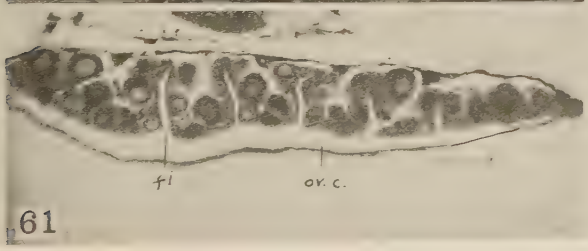
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PLATE 6

EXPLANATION OF FIGURES

62 Transverse section through the ovary of a 129-day female. × 85.

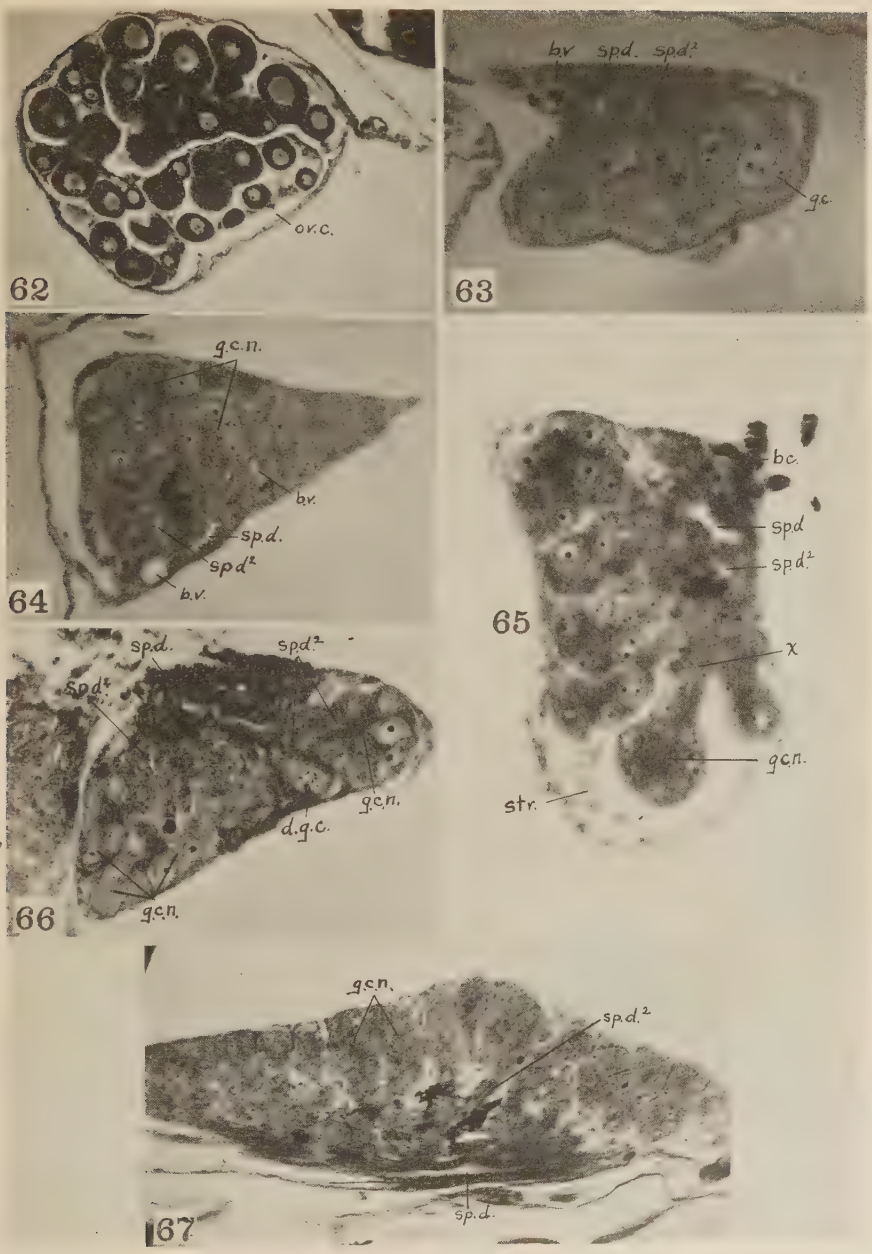
63 Transverse section through the testis of a fifty-nine-day male. × 395.

64 Transverse section of a testis from a seventy-three-day male. The side which is usually lateral lies dorsad. × 395.

65 Transverse section of a testis of a seventy-three-day male, showing early formation of tubules and secondary ducts. × 380.

66 Transverse section of a testis from a ninety-day male. × 395.

67 Longitudinal section of a testis from a 104-day male. × 185.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ov.c.
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sp.d.
sp.d.²
g.c.
64
g.c.n.
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bc.
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sp.d.²
x
g.c.n.
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66
sp.d.
sp.d.²
sp.d.²
g.c.n.
d.g.c.
g.c.n.
67
g.c.n.
sp.d.²
sp.d.

PLATE 7

EXPLANATION OF FIGURES

68 Section of an ovary from a 43-mm. female on July 4th. × 85.

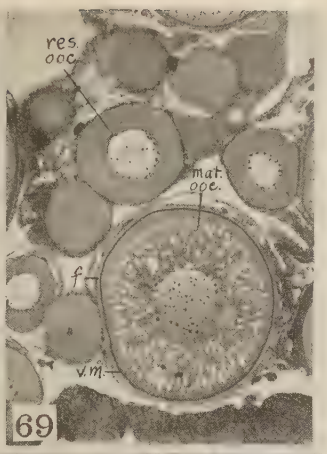
69 Section of an ovary from a 84-mm. female on August 27th. × 85.

70 Section of an ovary from a female fourteen months old, showing oogonial division and synizesis stages. × 395.

71 Section of an ovary from a female one year old. × 85.

72 Section of an ovary immediately after spawning. × 43.

mat. ooc.
68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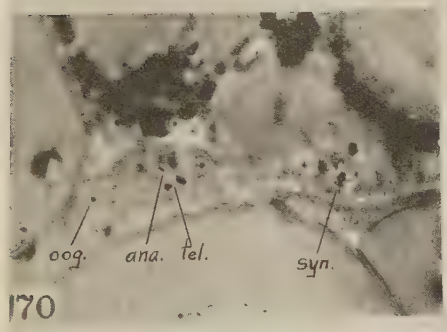
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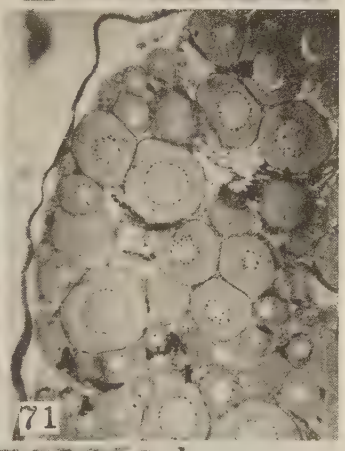
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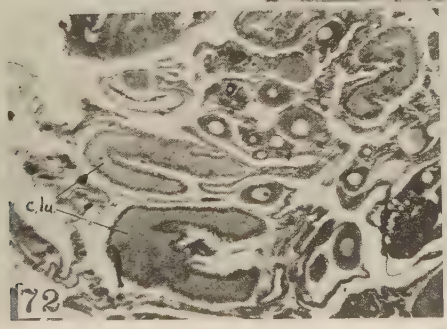

c.lu.
72

PLATE 8

EXPLANATION OF FIGURES

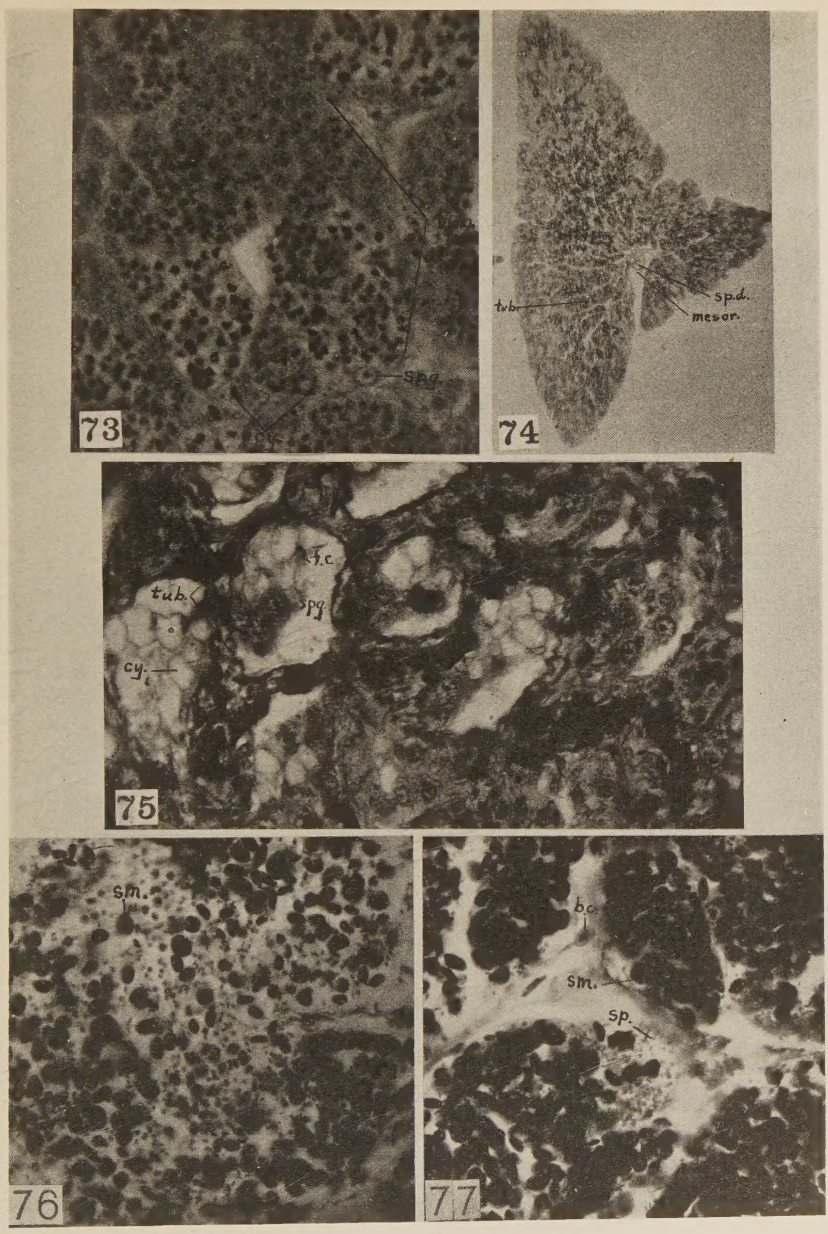
73 Section of a testis on September 19th, showing the cysts within the tubules. × 395.

74 Transverse section of a testis from a 71-mm. male on December 22nd. × 17.

75 Section of a testis two months after spawning, showing the spermatogonia within the old tubules. × 395.

76 Spermatid masses on February 1st, during formation. × 395.

77 Spermatid masses on April 1st, full size. × 395.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